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Ph.D. Thesis in Agricultural and Food Sciences

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Plant-litter feedback dynamics in terrestrial and aquatic ecosystems

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Authorship statement

I declare that this Ph.D. thesis is composed by scientific works carried out by myself in collaboration with my supervisors S.M., G.B and co-workers specialized in particular scientific topics.

1. Chapter 1 Synopsis. The general research question and its general scientific perspective were proposed by my supervisor S.M. I delineated the research question, described how it fits in the current scientific literature and described its potential impact.

2. Chapter 2. I contributed to the writing, data analysis and graphical improvement of work. The work has been published in the international journal Soil Biology and Biochemistry in 2018.

3. Chapter 3. I ideated and write the whole review at point 3.1. At point 3.2 of the thesis I defined the research question, proposed the methodology, discussed the methodology with my supervisors, made the experiments. Following, I made the data analysis and write the manuscript. Both works are unpublished.

4. Chapter 4. My co-supervisor G.B. proposed me the topic, the review at point 4.1 has been ideated and written by myself, in exception of a section including mathematical modelling written by N.S. The work is unpublished. The experimental work at point 4.2 was proposed by my co-supervisor G.B. He defined the research question and methodology. I made experimental work, F.D.F and Prof. D.E help me in molecular and bioinformatic analysis. I made data analysis, and work with manuscript structuration and writing. My supervisor revise it and I made correction. The work is accepted with major revision in New phytologist. The work at point 4.3 has been ideated by myself and proposed to Prof. F.G.A that supported my scientific questions. I made molecular and chemical analysis, bioinformatics, multivariate data analysis and written the manuscript. Work is unpublished. The work at point 4.4 was ideated by myself. I proposed the experiment to S.A.G the helped me in laboratory work, I write the manuscript and made data analysis. Work is unpublished.

5. Chapter 5. The work has been ideated both by my co-supervisor G.B. and me. We proposed the experiment to my supervisor S.M that accepted our experimental approach. I proposed field methodologies adopted in the work, GB suggested analytic methodologies, field work was carried out by me in collaboration with G.C., T.S. and M.I. me and GB made data analysis and write the work. Work is unpublished.

6. Chapter 6 Conclusion chapter has been written by myself. My supervisor S.M and my co-supervisor G.B. approved the manuscript.

6. Date:

7. Signature PhD candidate:

8. Signature promotor for agreement:

"There's something satisfying about getting your hands in the soil."

— E.A. Bucchianeri, Vocation of a Gadfly

Summary

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1. Synopsis

1.1. Overview on plant community ecology and plant-soil feedback.

Plant communities are dynamic biological entities. Their formation, decline and succession take place over a wide range of time scales in different biogeographical areas (Watt, 1947). A large body of literature has been published in the last century of research with the attempt to describe plant community dynamics in terrestrial ecosystems, showing that the magnification of these patterns relies on the interplay between environmental drivers and plant evolutionary strategies (Czaran & Bartha, 1992; Cadotte *et al.*, 2009; Pavoine *et al.*, 2011; Jiménez-Alfaro *et al.*, 2016). The processes of formation, transformation and successional substitution in plant communities are described in a disparate number of ecosystems such as woodlands, grasslands, riparian and coastal areas and involve interactions changing at different spatial scales, from macro-ecological gradients to site-specific environmental shifts (Bazzaz & Pickett, 1980; VAN ANDEL *et al.*, 1993; Cesarano *et al.*, 2017b). Accordingly, the objects of community dynamic assessment can range from the interplay between micro-environmental factors and seed germination or propagule establishment, to the environment-mediated feedback effects of a plant species presence on the structure and diversity of a plant assemblage (Watt, 1947; Mazzoleni *et al.*, 2010). Several theories explaining plant community diversity were previously proposed (Chesson, 2000). Among the most cited, shared and criticized theories, i) the intermediate disturbance hypothesis (IDH) states that dynamics in plant community are dependent by intermediate level of perturbation promoting the establishment of a species that otherwise would normally be outcompeted by dominant ones (Fox, 1981; Wilkinson, 1999; Bongers *et al.*, 2009; Catford *et al.*, 2012); ii) niche partitioning theory (Finke & Snyder, 2008), relies on the principle of competitive exclusion (Hardin, 1960), in which species are more likely to coexist when they tend to compete less strongly, thereby differentiating their niches by e.g. occupying different favourable locations or exploiting different resources avoiding overlap with other species (Cox, 1981; Ashton *et al.*, 2010); iii) the negative density dependence (NDD) hypothesis predicts that high local diversity, such as e.g. in tropical forests, is maintained by negative interactions at increasing density of conspecifics or between closely related plant species (Antonovics & Levin, 1980; Comita *et al.*, 2010). The so-called Janzen-Connell distribution, independently proposed half-a-century ago by D.H Janzen (1970) and J.H. Connell (1971) is a classic example of NDD, showing a decrease of recruitment near fruiting conspecific trees in tropical forest, traditionally attributed to host-specific microbial pathogens or herbivores (Clark & Clark, 1984; Hyatt *et al.*, 2003; Petermann *et al.*, 2008; Mangan *et al.*, 2010). iv) autotoxicity hypothesis relating the occurrence of plan-soil negative feedback to the accumulation of organic compounds released by the decomposition of plant litter (Mazzoleni *et al.*, 2007).

Over the last decades, the application of these theoretical frameworks to the variations observed in plant communities has repeatedly induced diverging points of view and reciprocal exclusion between different ecological thoughts (Silvertown & Law, 1987; Fox, 2013; Huston, 2014).

More recently, the needs of a solid and unitary concept explaining patterns of plant species distribution brought ecologists to the formulation of new conceptual frameworks, where plants are considered at different levels of biological organization and the soil as an interactive matrix in which vegetal communities develops.

Moreover, the need to explain patterns of plant establishment beyond the limitations imposed by differences in ecosystem characteristics led to the formulation of the plant-soil feedback framework (Bever *et al.*, 1997). According to such conceptualization, a plant is capable to induce changes in the soil, at both biotic and abiotic levels, which in turn affect the plant progeny and hence the community to which it belongs (Van der Putten *et al.*, 2013). The feedback interactions can favour the establishment of heterospecific plants in the same soil space, or being favourable for a conspecific. The two cases, as explained, are known as negative plant soil feedback (as being detrimental for the future generation of the conditioning species) and positive plant soil feedback (as favouring the propagation of the conditioning species), respectively. Accordingly, a negative direction of the feedback promotes establishment of different species and hence the formation of species-rich plant communities (Bonanomi *et al.*, 2005; Mazzoleni *et al.*, 2010). On the other hand, positive feedback enhances the proliferation of conspecific seedlings, leading to communities dominated by one or few species. This is also the case if the negative feedback is continuously removed as happening in riparian vegetation such as mangrove forests (Mazzoleni et al. 2010)

In the frame of plant diversity theories, the axiomatization of such interaction loops provides clear explanations for some previously observed patterns of plant community organization, consistent with niche partitioning as well as negative density dependence principles (Kulmatiski *et al.*, 2008; Van der Putten *et al.*, 2013). For instance, plant soil negative feedback can explain plant community successional patterns, where established plant species or assemblages can progressively lose competitive ability as a consequence of their conditioning effects on the soil substrate, leading to several species replacements along the successional dynamics (Kardol *et al.*, 2006; van de Voorde *et al.*, 2011). As such, the establishment of a new plant community would be the emergent property of a dynamic system undergoing negative feedback effects (Mazzoleni *et al*. 2010). Moreover, considering a plant community as a set of sub-communities, plant soil negative feedback can explain the cyclic disappearance and reappraisal of annual species in grassland (van der Maarel & Sykes, 1993; Vincenot *et al.*, 2017). Such process is known as carousel distribution model and predicts that the negative conditioning locally built-up by plants to the underlying soil, acts at short time scale leading to the spatial displacement of the future community within zones characterized by sub-community replacement. Finally, the plant soil feedback concept consistently explains different plant distributional patterns at population and individual scale, such as the characteristic ring-like configurations of different clonal plants (Cartenì *et al.*, 2012; Bonanomi *et al.*, 2014). These plants generally form ring-like patterns after central die-back and incapability to recolonize the already conditioned soil. Preferably, the newly formed vegetative portion of the plant colonize unconditioned soil resulting in an outward migration from the older central zone (Cartenì *et al.*, 2012; Bonanomi *et al.*, 2014). The concept at individual level indicate that negative plant soil feedback involves self-interactive processes that determine the spatial organization of the plants themselves. This was finely proposed in agricultural context (where species-specific plant-soil negative feedback conceptually correspond to the well-known soil-sickness in agronomy) suggesting that root proliferation in soil is a self-repulsive process (Zucconi, 2003). This rationale proposed by Zucconi is particularly suggestive within the conceptual framework of plant-soil negative feedback in different contexts. First, it suggests important socio-economical implications in crop

productivity, with promising eco-friendly applications to agricultural crops; second, under an evolutionary perspective, it suggests that negative plant-soil feedback was not positively selected, but rather intrinsically constitutive of the plant-soil system, as still holding notwithstanding the pluri-millennial selective pressure of agricultural practices aimed to increase crop yield; third, it may also explain the self-repulsive organization of plant organs in the surrounding environment that is widespread in plant kingdom. In this perspective, observations and conceptualizations derived from the agricultural research field, fits well to interpret negative plant-soil feedback effects in natural plant communities. Indeed, the need for a transdisciplinary conceptual framework for plant-soil feedback research has been recently pointed out by Mariotte *et al.* (2018).

In this context, the discovery that fragmented extracellular self-DNA (i.e. DNA originating from conspecifics) produces species-specific inhibitory effects recently demonstrated by Mazzoleni et al. (2015) provided a new frame for the mechanism of plant-soil negative feedback in both agricultural and natural ecosystems. Indeed, laboratory experiments reported that the inhibitory effect of purified conspecific DNA was evident on seed germination and root growth in both vitro- and soil- grown plants (Mazzoleni *et al.*, 2014). Second, the self-DNA inhibitory effect was found to be a generalized biological phenomenon by testing on several taxa including bacteria, protozoa, algae, fungi, and insects (Mazzoleni *et al.*, 2015b).

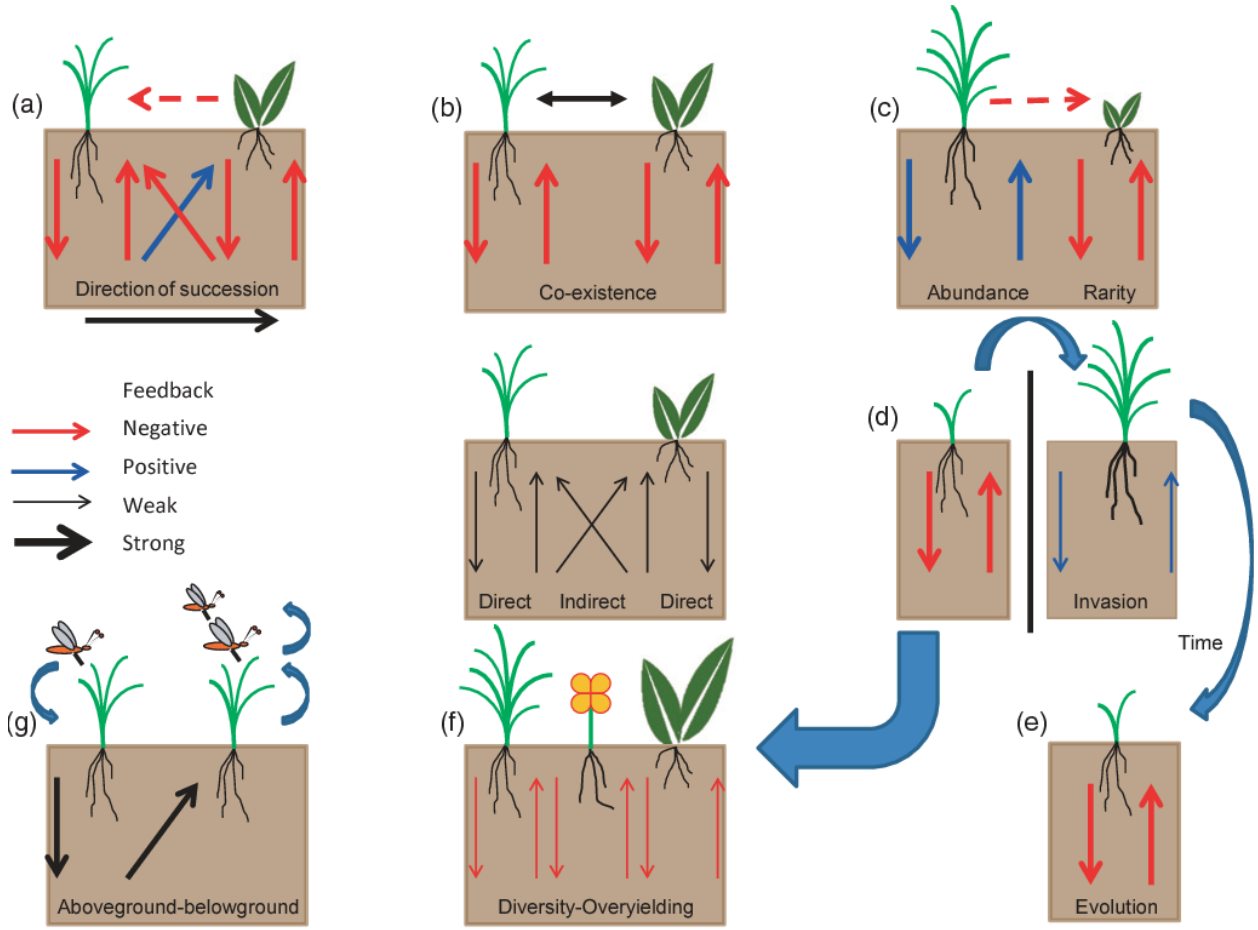

Figure 1.1 Conceptualized contribution of plant-soil feedbacks to plant community organization. From: van der Putten et al. (2013). Central panel: plant direct and indirect feedback effects to themselves and neighbours, respectively; (a) species replacement contributing to (primary or secondary) succession. (b) plant species coexistence; (c) combination of positive and negative soil feedback resulting in species abundance and rarity; (d) invasive species changing from negative to positive plant–soil feedbacks moving from native to introduced range; (e) over time, plant–soil feedbacks in the introduced range may become increasingly negative; (f) mild plant–soil feedbacks in mixed plant communities opposite to monocultures; (g) above- and below-ground feedback through herbivory-induced changes in the soil, influencing the subsequent plants, their aboveground herbivores, and the enemies of those herbivores. More details in the cited paper.

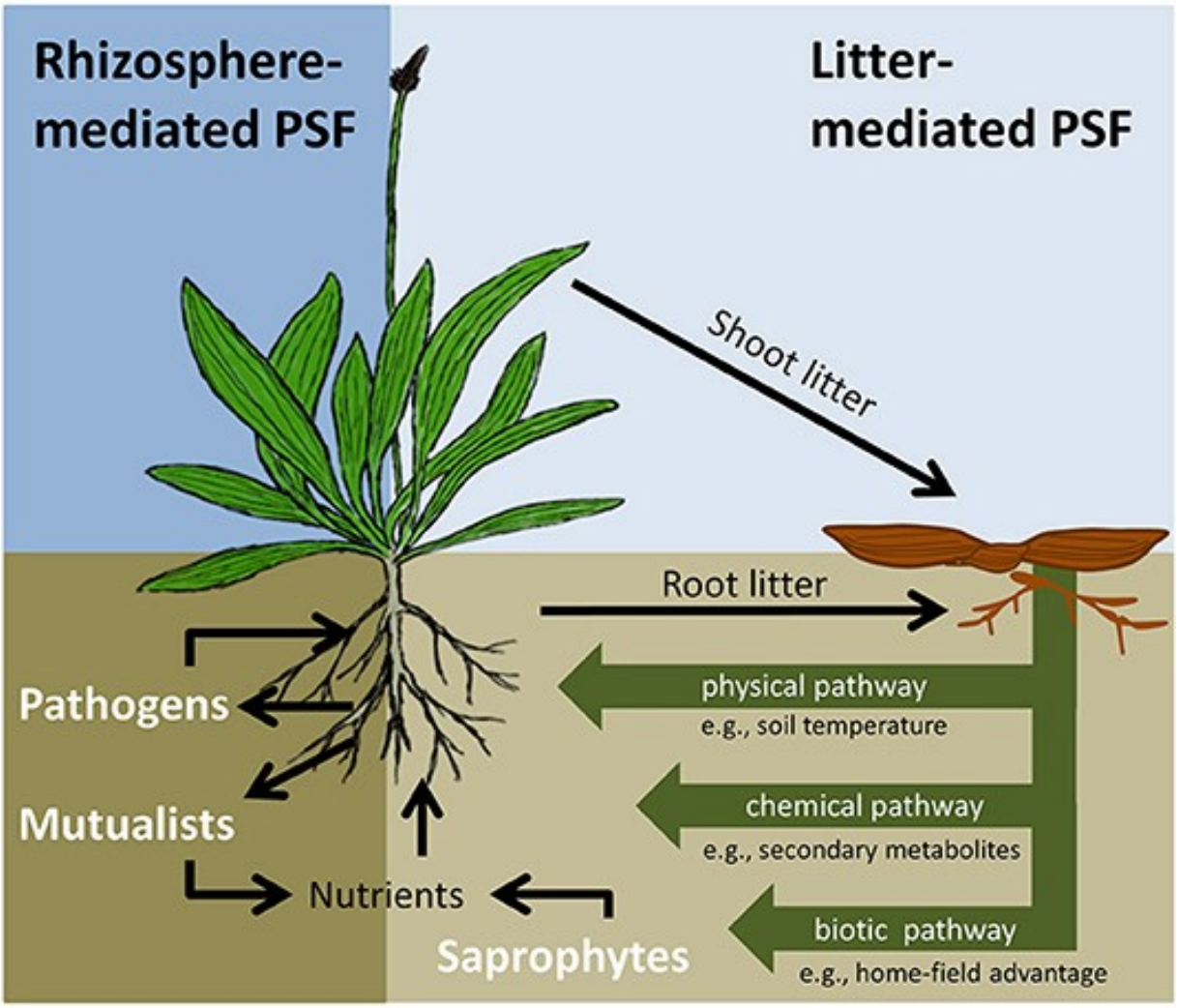

Figure 2.2: Overview of the plant-soil feedback framework integrating rhizosphere- and litter-mediated effects, driven by direct interactions between living plant roots and pathogens or mutualists, and by physical (e.g., litter layer thickness, litter physical traits), chemical (e.g., nutrient availability, secondary metabolites, allelopathy) or biotic (e.g., soil community composition, biotic interactions, home-field advantage) pathways. From: Veen et al. (2019). More details in the cited paper.

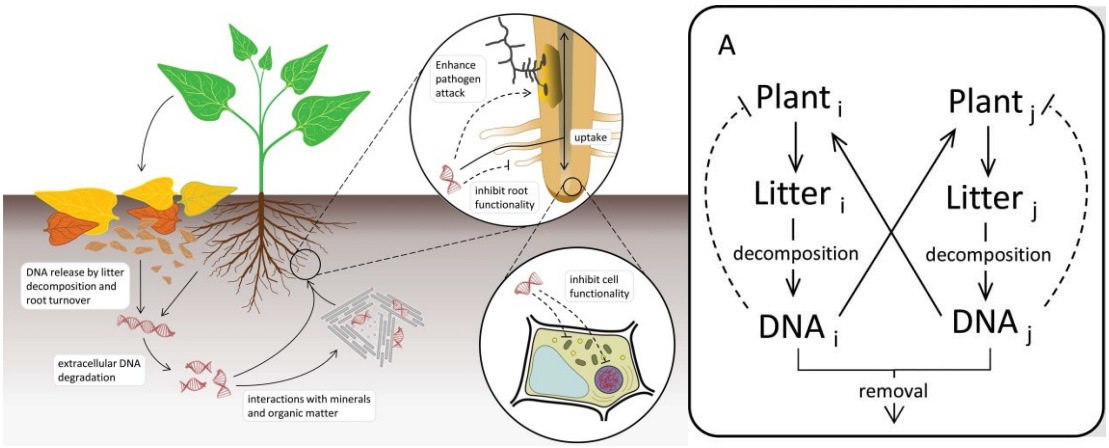

Figure 1.3: Conceptual representation of the self-DNA inhibitory effect as mechanism of species specific plant-soil negative feedback. From Carteni et al. (2016).

1.2. Mechanisms of plant-soil negative feedback.

The theoretical framework of plant-soil feedback has proven to be a powerful tool for scientists involved in the study of plant ecology worldwide. On the other hand, when focusing on negative feedback, that is apparently more common than the positive (Kulmatiski *et al.*, 2008; Cesarano *et al.*, 2017b), the conditioning effect of the plant on soil can produce substantially the same results despite originated by different mechanisms, as related to the manifolds interactive processes occurring belowground. Indeed, such interactions involve not only a wide variety of abiotic factors, but also multiple scales of biological organization. Different underlying mechanisms of plant-soil negative feedback have been proposed, but their relative importance in different contexts has not yet been clarified (Mazzoleni *et al*. 2015a;(Bennett & Klironomos, 2019; Veen *et al.*, 2019). Therefore, three main mechanisms are hereafter overviewed, with awareness that they are not the only ones.

One mechanism speculate that plant soil feedback is originated by nutrient limitation and niche depletion (Bonanomi *et al.*, 2008; Smith-Ramesh & Reynolds, 2017). The process well includes the niche patritioning theory and suggests that a focal plant species exploits nutrients available in the trophic niches it occupies. The nutrient immobilization in plant biomass bears two main consequences: first, nutrients are unavailable for plant species sharing the same trophic niche (including conspecific), which may be competitively excluded; second, the conditioning effect of the focal plant species modified the extant trophic niche, being potentially beneficial for heterospecifics with different nutritive requirements.

Another documented mechanism of plant soil negative feedback is that involving pathogen accumulation (Mills & Bever, 1998; Packer & Clay, 2000). Such process acts at both spatial and temporal scales and intermixes with species accumulation theories. Concerning spatial scale, a continuous monospecific spread corresponds to a metrical increase in the area occupied by a given species. Such trend leads to an increased probability for the species to encounter a natural enemy in its pattern of expansion or attracted from contiguous soil. In analogy to pathogen accumulation with spatial scale, the probability to accumulate enemies in the soil increases with plant age during its lifetime. The pathogen accumulation effect makes the conditioned soil inhospitable for the future generations or the extant population of conspecifics. As a consequence, the bottleneck

created by the soil-borne enemy on the targeted population opens the possibility for species non-sensitive to that specific pathogens to colonize free soil spaces or induces competitive advantages for heterospecifics.

Finally, a third mechanism producing plant soil negative feedback is the release of allelopathic compounds in soil. Release of allelopathic compounds has multifunctional aspects for plants. Primarily, phytotoxic compounds are waste products of internal catabolic and metabolic pathways, hence discarded in the surrounding environment, and, secondarily, the different forms by which these compounds are released modulates cues and competitive relations in the external soil environment (Duke, 2003; Rice, 2012). The release of allelopathic compounds can produce different effects on plant communities, depending whether targeting at heterospecific or conspecific level the neighbouring plants. For example, the release of allelopathic compounds increases the competitive ability of the releasing species when the chemical compounds are directly toxic or able to interfere the activities of competitors (Rose *et al.*, 1984; Mallik, 1998). In other cases, the toxicity expressed by a plant can act at conspecific level in the surrounding environments (Singh, H *et al.*, 1999; Mazzoleni *et al.*, 2007; Mazzoleni *et al.*, 2015a).

All three mechanisms are identified as fundamental drivers of negative plant soil feedback. The attempt to establish their relative importance in different contexts is still matter of debate between plant ecologist. However, the onset of one of these processes is likely to enhance the appearance of another one, thus resulting in a cumulative effect (Bonanomi *et al.*, 2007).

1.3. Induction of phytotoxicity by litter decomposition

One of the most frequent mechanisms of plant soil negative feedback is connected to the release of allelopathic compounds in soil (Rice, 2012). As already mentioned, allelopathic interactions are originated from the deposition of organic matter in soil in form of root exudates and litter. In some case, these products showed non-specific and generalized impairment of growth for plants in their proximity. Posing the attention on leaf litter, inhibitory effects have been reported by a number of studies (Xiong & Nilsson, 1999) and are caused because litter acts as a physical barrier (Scarpa & Valio, 2008) or, more commonly, by a chemical interference effect (Rice, 2012). In this latter case, plant inhibition is the results of a combination of nutrient starvation and chemical toxicity due to allelopathic compounds. Nutrient starvation mainly involve nitrogen (N) and is caused by microbial competition when decomposing organic matter had a C/N ratio above the threshold values of ~30 (Hodge *et al.*, 2000). In presence of N poor plant leaf litter, large root or wood debris, microbe outcompete plants for mineral N uptake, physically mobilizing nitrogen into the organic matter (Lummer *et al.*, 2012) and, as a consequence, cause a deprivation of mineral N in the surrounding soil that may impair plant growth. The intensity and duration of N immobilization depends on the amount and C/N ratio of the considered litter, and may last from few weeks, as in case of leaf litter, to several years when large amount of wood is involved, as observed after disturbance (Zimmerman *et al.*, 1995). Direct litter phytotoxicity is also widespread, with three studies that reported an inhibitory effect for 21 (Lopez-Iglesias *et al.*, 2014), 64 (Bonanomi *et al.*, 2011) and 65 (Meiners, 2014) litter types in temperate and Mediterranean ecosystems. A wide array of phytotoxic compounds have been isolated and identified, especially in water leachate of fresh litter and during the early phases of decomposition (Rice, 2012). The most common phytotoxic compounds in litter include short-chain organic

acids as propionic and butyric acids (Armstrong & Armstrong, 1999), tannins (Kraus *et al.*, 2003), and low molecular weight phenols (Li *et al.*, 2010). In this regard, previous studies also clarified that litter phytotoxicity is largely affected by plant functional type and the stage of decomposition. In detail, leaf litter is usually more phytotoxic than root debris and, among plant functional types, tissues of nitrogen-fixing species are, on average, more inhibitory than forbs, woody species and grasses (Bonanomi *et al.*, 2017b). Moreover, it is also well established that a rapid transformation and degradation of most labile allelochemical compounds into non-toxic molecules causes a rapid disappearance, usually in the time-frame of weeks or few months, of the litter phytotoxic effect (Chou & Patrick, 1976; Bonanomi *et al.*, 2006a; Dorrepaal, 2007)**.**

A notable exception to this general pattern is the inhibitory effect exerted by litter of conspecifics. In this case, plant debris have a species-specific autotoxic effect (Singh, H *et al.*, 1999), with inhibition that is long-lasting, up to several years (Cesarano *et al.*, 2017b). Such species-specific and persistent autotoxicity has been associated to the persistence and accumulation of extracellular DNA (exDNA) in the litter layer and underlying soil during decomposition (Mazzoleni *et al.*, 2015a).

Overly, these studies provided evidence litter mediates plant community structuration (Facelli & Pickett, 1991). The effect on plant community is widely described due to the release of phytotoxic compound acting at species specific level during litter decomposition. In light of this, is intriguing to understand the way in which some communities can magnify in a monodominated pattern and which is the contribution of litter in originating plant soil positive feedback.

A thought-provoking example of is that of plant community in acquatic ecosystems. In these kinds of assemblages is common to find monospecific communities. Floating plants (*Eichornia crassipes, Lemna spp., Pistia spp.*), perennial species in wetlands and marshes (*Phragmites australis, Spartina spp., Typha spp.*), gallery (*Mora spp., Tabebuia spp.*) and mangrove forests (*Avicennia spp., Nypa fruticans, Rhizophora spp.*), and also seagrass (*Posidonia spp., Thalassia spp., Zoostera spp.*), seaweed and kelp forests (*Fucus spp.*, *Laminaria spp.*, *Macrocystis pyrifera etc*) are low diversity phytocoenosis find in both, salt and freshwater condition and latitudinal level. In these cases, despite the absence or limited presence of soil, the conditioning effect of the plant on its own substrate appear to be undefective, maybe because removed by the motility of water that is able to displace phytotoxic agents from the proximity of plant or dilute them until ineffective concentrations (already proposed by Mazzoleni at al. 2007).

Another useful example is that of monodominated forests in boreal, temperate and sporadically in tropical belts (Hart *et al.*, 1989; Corrales *et al.*, 2018). These community are normally dominated by a single plant species and are characteristic of the majority of the forested lands of the word (Brundrett, 2004). The low diversity of this community indicated that dominant plant species provide a positive conditioning of soil for conspecific renewal increasing their probability of survival. On the other side, classic negative feedback experiment demonstrated that seedlings of that trees growing in soils conditioned by conspecific has suppressed growth (Wurst *et al.*, 2015). This because those experiment scrutinized the effect of a conspecific conditioned soil on plant considering it as a singular biological entity, but not considering it as an holobiont units composed by more than one organism. As in parallel, actual insight in biomedical sciences consider that human organisms

are composed also by the microbiome hosted in the gastrointestinal tract, the missing piece for consideration of plant as a holobionts is the presence of their elective symbionts in plant root system (Simon *et al.*, 2019; Thomashow *et al.*, 2019). Indeed, monodominated forests are all characterized by the ability to form ectomycorrhizal symbiosis (Corrales *et al.*, 2018). In this kind of relationship a mutualistic exchange of nutrients between the two counterparts modify the competitive relations between plant in community, favouring plants able to forms symbiotic relationships (Brundrett, 2004). The ability of ectomycorrhizal plants to persist to the effect of negative feedback was clearly described in experiment including ectomycorrhizal partner (Bennett *et al.*, 2017; Teste *et al.*, 2017). However, evidence that the recurrence of these symbiotic partner allows plants to exploit some fungal strategy to escape from autotoxicity released by its own litter is not present in literature.

1.4. Litter decomposition mediate microbial dependent plant-soil feedback

In the cases observed, the release of phytotoxic compound from litter induce effects of variable intensity and direction for plants and this is dependent by the decomposition stage of the organic matter. These effects could take place and affect plant growth: i) with fresh litter being generalized detrimental; ii) with decomposed litter, that could be detrimental as well as in the case of autotoxic self-DNA; iii) again with decomposed litter, but positive for heterospecific plant.

Intriguingly, the results of the litter deposed by plants, and successive decomposition, could provoke the formation of particular microbiomes that in turn modify the structure of a stable plant community. During the process of litter decomposition, a series of microbial communities succeed according to the variation, depletion and formation of different trophic niches in a time series (Purahong *et al.*, 2016; Bonanomi *et al.*, 2019). Whether at the beginning of litter decomposition the microbial community increasing in abundance is specialized on the depletion of labile and firstly available compounds, the successive communities replace the first because of the rise of a new resource and because the first community suffer the exploitation of resources in the former niche. This process is continually repeated until the complete exhaustion of the decomposed substrate. Moreover, the successional series of communities will follow an increased gradient of recalcitrant property of the resources. Within the set of community formed has been assessed that those occurring at later stages evolved a wider set of strategies in order to scavenge resources. The most commonly example is that described for communities of Basidiomycetes fungi (Frankland, 1998). Most of Basidiomycetes are C-selected species with respect to other fungal phyla that are mainly composed by R-selected and S-selected taxa (Hiscox *et al.*, 2018). The selective strategies for fungi suggests that C-selected species are characterized by slow growth rate, ability to adapt to diverse trophic resources and combative behavior with respect to competitors (Dix, 2012; Boddy & Hiscox, 2016). In physiological terms, the description of the C-selected strategies among fungi means that the species is able to defend itself and its resources through the synthesis of complex molecules overcoming the disadvantages from the dependence of a single trophic resources. This is possible by the development of a wider arsenal of degrading enzymes (Baldrian, 2008; Spiteller, 2008). Presumably, the high energetic costs of this evolutive development leads Basidiomycetes to growth with slower replicative rates and, hence occurs in later stage of decomposition of the substrates (Frankland, 1998; Osono & Takeda, 2001a). The domination of the soil microbial community operated by Basidiomycetes has effect on the extant plant

community. In the case this process occurs in woodland floor the community is often dominated by the aforementioned ectomycorrhizal guild leading to monodominated distribution of plant species (as previously stated). In the case the process take place in grassland ecosystems, with fungi belonging to saprobic Basidiomycetes guilds, the whole decomposition process can lead to the formation of patterns of changing vegetation also known as fungal fairy rings (Shantz & Piemeisel, 1917). Particularly, these processes are detrimental and, successively, stimulant for the plant communities. Moreover, the powerful interaction produced by the dominant basidiomycetes can include it in the list of the ecosystem engineer species because reshaping the structure of a plant community (Bonanomi *et al.*, 2012).

1.5. Aims and contents of the thesis

The present thesis has the propose to extend the analysis of the occurrence of the phenomenon of plant soil feedback derived by the litter decomposition processes. Despite the wide variety of forms in which plant-soil feedback interaction could manifest, the works composing the thesis are addressed to shed light on particular mechanisms that bring to low diversity plant community. Moreover, is proposed here to describe the extent to which modification of the microbial community, derived from the process of litter decomposition, can affect the structure of plant communities. In order to accomplish these challenging claims, the scientific works composing the present document were conducted in different natural conditions and at different biological level. The ecosystem in which these processes are studied in the present thesis are:

1- The monodominated ectomycorrhizal forests, where the interplay between litter, mycorrhizal symbiosis and the presence of a microbial decomposing community in soil has been studied to assess the modulation of plant-soil feedback. The study of how ectomycorrhizal symbiosis produce advantages for monodominant plants has been realized by a classic biomass-based approach and later to elucidate the advantages produced by the relation between litter decomposition and the associated autotoxicity with the ectomycorrhizal symbiosis by assessing pattern of colonization of roots in soil.

2- The grassland ecosystem to study the magnification of the effect of microbial dominance in the later stage of litter decomposition and the effect on extant plant community. In particular, the relationship between microbial dominance dynamics and vegetation was assessed in the context of occurrence of "fairy rings" created by Basidiomycetes fungus.

3- Water ecosystems to assess the direct effect of litter phytotoxicity on plants without the impediment characterizing the complex soil matrix. This experimental setting was conceived to get insights on the inhibitory action of extracellular self-DNA inducing autotoxicity.

Additionally, the first chapter of the thesis reviews how microbial succession works according to different litter species and the changes induced in microbial communities acting on it.

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2. Linking bacterial and eukaryotic microbiota to litter chemistry: Combining next generation sequencing with ^{13}C-CPMAS-NMR spectroscopy

Overview

Microbial succession over decomposing litter is controlled by biotic interactions, dispersal limitation, grazing pressure, and substrate chemical changes. Recent evidence suggests that the changes in litter chemistry and microbiome during decomposition are interdependent. However, most previous studies separately addressed the microbial successional dynamics or the molecular changes of decomposing litter. Here, we combined litter chemical characterization by ^{13}C NMR spectroscopy with next generation sequencing to compare leaf litter chemistry and microbiome dynamics using 30 litter types, either fresh or decomposed for 30 and 180 days. We observed a decrease of cellulose and C/N ratio during decomposition, while lignin content and lignin/N ratio showed the opposite pattern. ^{13}C NMR revealed significant chemical changes as microbial decomposition was proceeding, with a decrease in O-alkyl C and an increase in alkyl C and methoxyl C relative abundances. Overall, bacterial and eukaryotic taxonomical richness increased with litter age. Among Bacteria, Proteobacteria dominated all undecomposed litters but this group was progressively replaced by members of Actinobacteria, Bacteroidetes, and Firmicutes. Nitrogen-fixing genera such as *Beijerinckia* and *Rhizobium* occurred both in undecomposed as well as in aged litters. Among Eukarya, fungi belonging to the Ascomycota phylum were dominant in undecomposed litter with the typical phyllospheric genus *Aureobasidium*. In aged litters, phyllospheric species were replaced by zygomycetes and other ascomycetous and basidiomycetous fungi. Our analysis of decomposing litter highlighted an unprecedented, widespread occurrence of protists belonging to the Amebozoa and Cercozoa. Correlation network analysis showed that microbial communities are non-randomly structured, showing strikingly distinct composition in relation to litter chemistry. Our data demonstrate that the importance of litter chemistry in shaping microbial community structure increased during the decomposition process, being of little importance for freshly fallen leaves.

2.1. Introduction

Decomposition of leaf litter is a fundamental process for biogeochemical cycles in terrestrial ecosystems, controlling the formation of organic carbon (C) and nutrient stocks and, then, affecting above- and below-ground ecosystem functioning and community composition. At global and regional scales, litter decomposition are primarily controlled by climatic factors as temperature and water availability (Aerts, 1997b), whereas at community level litter chemistry plays a pivotal role (Melillo *et al.*, 1982; Cotrufo *et al.*, 2013).

Leaf litter supports microbiome activity providing organic carbon and nutrients (Hättenschwiler *et al.*, 2005). A large number of studies investigated the dynamic changes of bacterial and fungal community composition during decomposition of leaf litter (Hudson, 1968; Dix & Webster, 1995). Several mechanisms have been proposed to explain the observed community shifts, including inter-specific competition, dispersal limitation (Frankland, 1998), time constraints of fungal fruiting body development (Harper & Webster, 1964), grazing pressure of arthropods (Newell, 1984) and, most important, chemical changes of substrate during decomposition (Osono & Takeda, 2001b; Rinkes *et al.*, 2011). Similar to what has been described for animals and humans, where gut microbiome composition is shaped by the dietary habits e.g (Bäumler & Sperandio, 2016; De Filippis *et al.*, 2018), the amount and quality of leaf litter play a key role in defining microbiome composition and functions. In this regards, it is crucial highlighting that heterotrophic microbes, by progressively exploiting leaf litter, continuously alter its molecular composition (Swift *et al.*, 1979; Berg & McClaugherty, 2008) that, in turn, controls microbial turnover. For instance, the rapid disappearance of the so called "sugar fungi" replaced by cellulolytic and ligninolytic fungi, is a well-recognized successional pattern (Hudson, 1968; Frankland, 1998), associated with the depletion of labile C compounds during decomposition. In this context, an accurate definition of litter chemistry is crucial to understand the connection and interaction between litter type, decomposition and microbiome composition and dynamics (Bhatnagar *et al.*, 2018).

Litter chemistry dramatically varies among species across and within ecosystems, depending on the plant functional type and leaf traits (Cornwell *et al.*, 2008). Moreover, during microbial decomposition, mainly driven by fungi and bacteria, the chemistry of the organic substrate continuously changes. Different litter chemical fractions decompose at different rates, with the rapid depletion of labile C compounds leading to relative preservation of more recalcitrant compounds such as lignin (Berg & McClaugherty, 2008). Microbes progressively transform organic materials into novel recalcitrant materials that accumulate as decomposition proceeds (Preston *et al.*, 2009b). Historically, C/N and lignin/N ratios have been the most widely used indicators of litter chemistry (Melillo *et al.*, 1982; Taylor *et al.*, 1989). However, recent studies demonstrated the limited usefulness of C/N and lignin/N ratios to predict litter impact on different ecosystem functions, including litter mass loss (Bonanomi *et al.*, 2011), phytotoxicity towards root proliferation (Mazzoleni *et al.*, 2015a), aggregate structural stability (Sarker *et al.*, 2018), and organic C-cycling (Incerti *et al.*, 2017). Searching for finer chemical predictors, several studies based on ^{13}C-cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (hereafter ^{13}C NMR) clarified the major molecular changes experienced by ageing litter, including a rapid decrease of amino acids, polypeptides, and *O*-alkyl and di-*O*-alkyl C fractions

corresponding to carbohydrates, and a relative enhancement of aliphatic C fraction, associated with microbial spoilage, and aromatic, methoxyl and *N*-alkyl C fractions diagnostic of lignin content (Kögel-Knabner, 2002).

Taken together, available evidence indicates that litter chemistry is likely a key driver of bacteria, fungal and protist community dynamics. Indeed, a growing number of studies, based on molecular methods, have recently described the complex dynamics of bacterial and fungal communities that co-occurs and functionally interacts during the course of litter decomposition (Schneider *et al.*, 2012; Voříšková & Baldrian, 2013). However, the majority have separately addressed either the microbial successional dynamics, or the molecular changes of decomposing litter, while attempts to simultaneously target both topics over a wide range of litter types are still lacking. At most, some recent studies described the microbiome of one or few litter types (Purahong *et al.*, 2016; Tláskal *et al.*, 2016), as related to basic litter chemistry parameters (i.e. C/N and lignin/N ratios). Such approach, based on heavily criticized indicators of litter chemistry (Cartenì *et al.*, 2018) does not allow an exhaustive description of the substrate molecular dynamics, which heavily limits the inference drawn from the experimental results. Aimed to overcome such limitations, we combined powerful organic chemistry and molecular biology tools with next generation sequencing to compare leaf litter chemistry and microbiome dynamics. In particular, we fully characterized the bacterial and fungal microbiomes and the relative abundance of C bond types in 10 litter types, either fresh or decomposed for 30 and 180 days. The main objectives of our study were:

(i) to assess successional differences of microbial communities over different litter types;

(ii) to describe litter chemistry dynamics using advanced molecular techniques (^{13}C NMR) as compared to basic parameters (C/N and lignin/N ratios);

(iii) to explore the relationships between the molecular properties of decomposing litter and the successional dynamics of bacterial and fungal communities.

2.2. Material and methods

Litter collection

A pool of ten plant species representing a wide range of litter quality were selected from Mediterranean and temperate ecosystems (Southern Italy). Such pool included a grass (*Festuca drymeia*), a perennial forb (*Acanthus mollis*), an evergreen shrub (*Coronilla emerus*), one vine (*Hedera helix*), two evergreen trees (*Pinus halepensis* and *Quercus ilex*), and four deciduous trees (*Fagus sylvatica*, *Fraxinus ornus*, *Populus nigra*, and *Robinia pseudoacacia*). Two species are nitrogen-fixing (*C. emerus* and *R. pseudoacacia*). For each species, at least 15 individuals from natural communities were randomly selected at the sampling sites. Freshly abscised leaves were collected by placing nets under the plants, carried to the laboratory, dried (at 40°C until constant weight was reached) and then stored in sealed plastic bags at room temperature. Considerable effort was maintained throughout sampling to ensure clean, uncontaminated leaf samples, including use of gloves during sample collection and decontamination of equipment prior to and during sampling.

Litter decomposition experiment

Litter decomposition was carried-out in microcosms in laboratory under controlled, optimal conditions, to isolate the effect of litter quality on microbiome composition, excluding effects of fluctuation of climatic conditions. The decomposition experiment was performed in microcosms following litterbag method (Berg & McClaugherty, 2008). In detail, large (20 x 20 cm^2) terylene litterbags (mesh size 1 mm) were filled with 5 g of undecomposed, dry leaf litter and placed in plastic trays (120 x 120 x 25 cm). Subsequently, we obtained natural source of microbial inoculum as described in Bonanomi et al. (2011). Briefly, the inocula was prepared by mixing 10 g of a single soil type taken from the fields in correspondence of each litter collection point (top 10 cm) and 90 g of water. After preparation, inoculum was sprayed over the litter-bag. The addition to litter of its corresponding soil inoculum was aimed to enhance the decomposition process and reproduce the microbial community available at the provenance soil at the beginning of the decomposition process. Microcosms were kept in a growth chamber under controlled temperature (18±2°C night and 24±2°C day) and water (watered every seven days to field capacity with distilled water) conditions. At each harvesting date (0, 30, and 180 days since the start of the experiment), 10 litterbags (replicates) were collected. Bags were dried in the laboratory (at 40°C until constant weight was achieved) and the remaining material weighed. In this way, 30 organic materials (10 species at three sampling points) with different ages were produced: fresh, undecomposed litter (thereafter indicated as 0 days) and litter decomposed for 30 or 180 days.

Litter chemistry

The 30 materials were chemically characterized by measuring total C and N contents (flash combustion of microsamples - 5 mg of dry, pulverized litter - Elemental Analyser NA 1500 - Carlo Erba Strumentazione, Milan, Italy) labile C, proximate cellulose and lignin content (Gessner, 2005) and spectral data from ^{13}C NMR in solid state under the same conditions in order to allow a quantitative comparison among spectra. (Spectrometer Bruker AV-300 - Bruker Instrumental Inc, Billerica, MA, USA). Analytical setup included 4 mm wide-bore magic angle spinning (MAS) probe, MAS of rotor spin 13000 Hz, recycle time 1 s, contact time 1 ms, acquisition time 20 ms, 2000 scans, sample packed in 4-mm zirconium rotors with Kel-F caps (Wilmad／Lab Glass, Buena, NJ, USA), pulse sequence applied with a 1H ramp to account for nonhomogeneity of the Hartmann–Hahn condition at high spin rotor rates. Each NMR spectrum was automatically integrated (iNMR software, www.inmr.net) to calculate the area of the peaks which appeared in the chosen region. Selection of spectral regions and identification of C-types were performed according to previous studies (Bonanomi *et al.*, 2015). The following seven regions and C types were considered: 0--45 p.p.m = alkyl C; 46--60 ppm = methoxyl C; 61--90 ppm = *O*-alkyl C; 91--110 ppm = di-*O*-alkyl C; 111--140 ppm = *H*- and *C*- substituted aromatic C; 141--160 ppm *O*-substituted aromatic C (phenolic and *O*-aryl C); 161--190 ppm carbonyl C.

DNA extraction and sequencing

Total DNA extraction from litter samples was carried out by using the PowerSoil DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions. The bacterial and eukaryotic diversity were assessed by pyrosequencing of the V1-V3 regions of the 16S rRNA gene (~520 bp) and a portion of the 18S rRNA gene (~436 bp), respectively. Primers and PCR conditions were previously

reported (Ercolini *et al.*, 2012; De Filippis *et al.*, 2017). PCR products were purified with the Agencourt AM-Pure kit (Beckman Coulter, Milan, IT) and quantified using a Plate Reader AF2200 (Eppendorf, Milan, IT). Equimolar pools were obtained prior to further processing and sequenced on a GS Junior platform (454 Life Sciences, Roche Diagnostics, IT), according to the manufacturer's instructions.

Sequence data analysis

Raw reads were filtered and analysed by using the QIIME 1.8.0 software (Caporaso *et al.*, 2010). Reads shorter than 300 bp, with more than 1 primer mismatch and with average quality score lower than 25 were discarded. OTUs were picked through *de novo* approach and uclust method and taxonomic assignment was obtained by using the RDP classifier and the Greengenes (McDonald *et al.*, 2012) or the Silva SSU/LSU rRNA gene database release 119 (Quast *et al.*, 2012). Chloroplast and Streptophyta contamination, as well as singleton OTUs, were removed and the relative abundance of other taxa was recalculated. In order to avoid biases due to the different sequencing depth, OTU tables were rarefied to the lowest number of sequences per sample. The 16S and 18S rRNA gene sequences are available at the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI), under accession number SRP151263.

Data analysis

Statistical analyses and plotting were carried out in R environment (http://www.r-project.org) and with Statistica 10.0 (STATSOFT, TULSA, OK). Alpha-diversity analysis was carried out in QIIME on rarefied OTU tables, calculating Chao's 1 estimator and Shannon's index (H).

Generalized Linear Mixed Models (GLMMs) were used to assess main and second order effects of litter species (treated as a random effects) and age (treated as a continuous covariate) on OTU relative abundance and alpha diversity parameters. Pairwise differences were tested for statistical significance using Duncan's post-hoc test at $P < 0.05$. Heatplots were generated by *made4* R package using hierarchical Ward-linkage clustering based on the Spearman's correlation coefficient of the bacterial or Eukaryotic taxa abundances.

Non-metric multidimensional scaling (NMDS) was carried out using the *metaMDS* function (*vegan* package) on a Bray-Curtis dissimilarity matrix calculated on bacterial or fungal genera abundance. Leaf litter chemical parameters were fitted to the NMDS ordination plots using the *envfit* function (*vegan* package). Spearman's pairwise correlations were computed between bacterial or eukaryotic phyla abundance and litter quality parameters (*corr.test* function in *psych* package). Correction of p-values for multiple testing was performed when necessary (Benjamini & Hochberg, 1995). Statistically significant correlation scores were visualized in a network using Cytoscape v. 3.4.0 (http://www.cytoscape.org), with nodes representing bacterial and eukaryotic phyla and litter elemental, proximate, and ^{13}C NMR-based chemical traits.

2.3. Results

Microbiome diversity

Litter age showed significant enhancing effects on OTU number and microbial diversity (Table 2.1; Figure 2.1), although with different magnitude for bacterial and eukaryotic communities. In all cases, bacterial and eukaryotic diversity was higher in litter decomposed for 180 days compared to fresh materials ($P<0.05$;).

However, OTU number and microbial diversity largely varied among litter species. In particular, a remarkable increase in bacterial OTUs with litter age was observed for *F. drymeia*, *P. halepensis* and *P. nigra* litters, while the same pattern in eukaryotic diversity was most evident for *F. sylvatica* and *R. pseudoacacia* litters. Much more rarely, OTUs number and microbial diversity decreased during decomposition, as in the case of Bacteria in *R. pseudoacacia* litter.

Table 2.1**:** Summary of the Generalized Linear Mixed Models (GLMMs) testing for main and interactive effects of litter materials on Bacteria and Eukarya community diversity metrics (i.e. number of observed OTUs, Chao's 1 estimator, Shannon's index). Tested effects included litter species (S, random categorical factor; ten levels) and age (A, considered as a continuous covariate). Significant P-values are in bold type.

	Bacteria							**Eukarya**					
	Effect type	**SS**	**df**	**MS**	***F***	***P***		**Effect type**	**SS**	**df**	**MS**	***F***	***P***
N of OTUs													
S	Random	21750.5	9	2416.7	0.76	0.6578	S	Random	4359.4	9	484.4	0.31	0.9524
A	Fixed	25671.0	1	25671.0	19.00	**0.0018**	A	Fixed	7698.2	1	7698.2	20.93	**0.0013**
S × A	Random	12157.2	9	1350.8	0.42	0.8948	S × A	Random	3309.8	9	367.8	0.24	0.9792
Chao's 1 estimator													
S	Random	69505	9	7723	0.65	0.7344	S	Random	20768	9	2308	0.47	0.8642
A	Fixed	252392	1	252392	33.20	**0.0003**	A	Fixed	33662	1	33662	19.14	**0.0018**
S × A	Random	68423	9	7603	0.64	0.7419	S × A	Random	15827	9	1759	0.36	0.9310
Shannon's index													
S	Random	4.57	9	0.51	0.39	0.9160	S	Random	4.83	9	0.54	0.33	0.9456
A	Fixed	3.09	1	3.09	7.93	**0.0202**	A	Fixed	3.90	1	3.90	8.34	**0.0180**
S × A	Random	3.51	9	0.39	0.30	0.9594	S × A	Random	4.21	9	0.47	0.29	0.9632

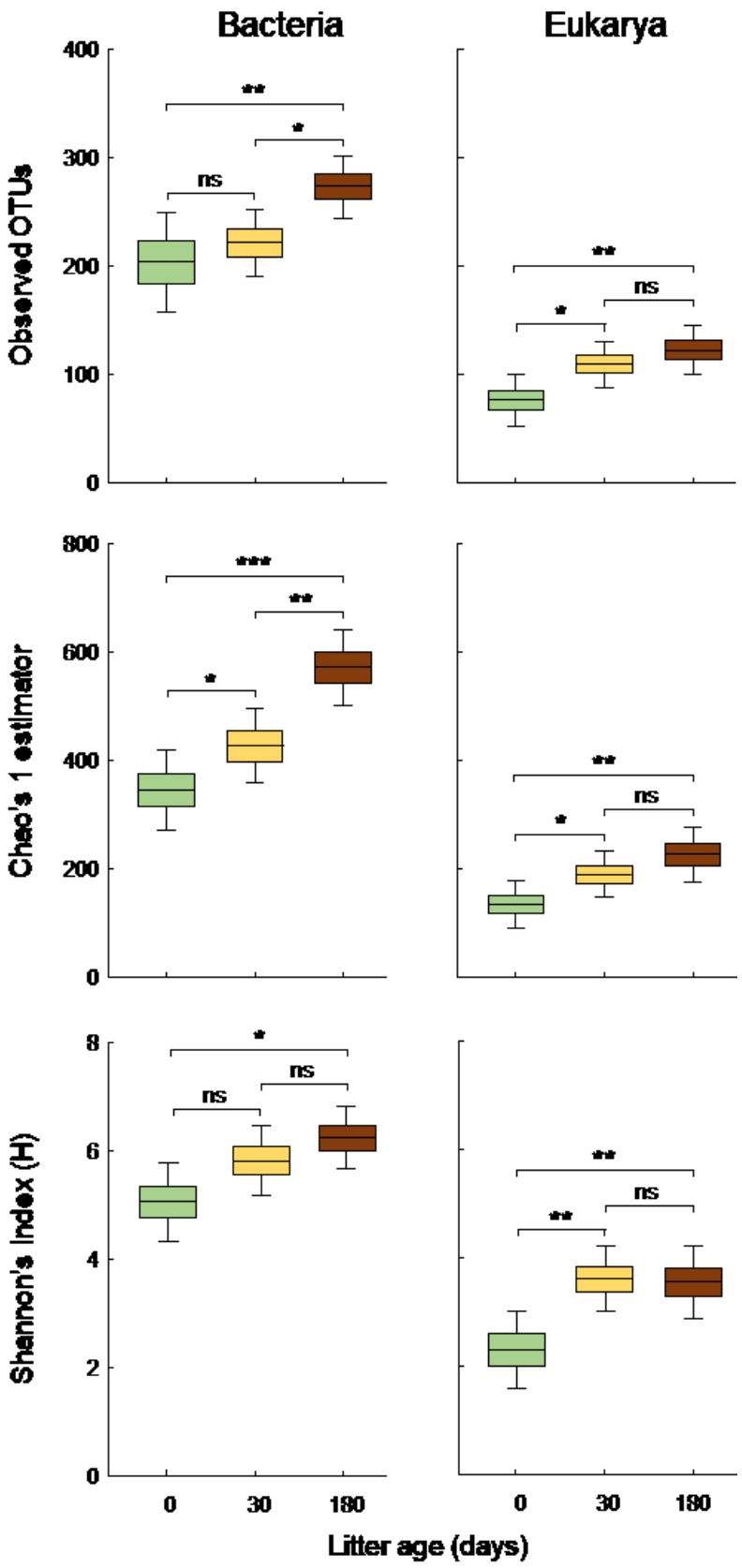

Figure 2.1: Number of observed OTUs, Chao's 1 diversity estimator and Shannon's index of Bacteria (left) and Eukarya (right) communities in litter materials of different ages. Within each panel, boxplot data refer to mean (line), standard error (box) and 95% confidence interval (whisker) of 10 litter materials at each litter age, with age-dependent differences indicated according to statistical significance.

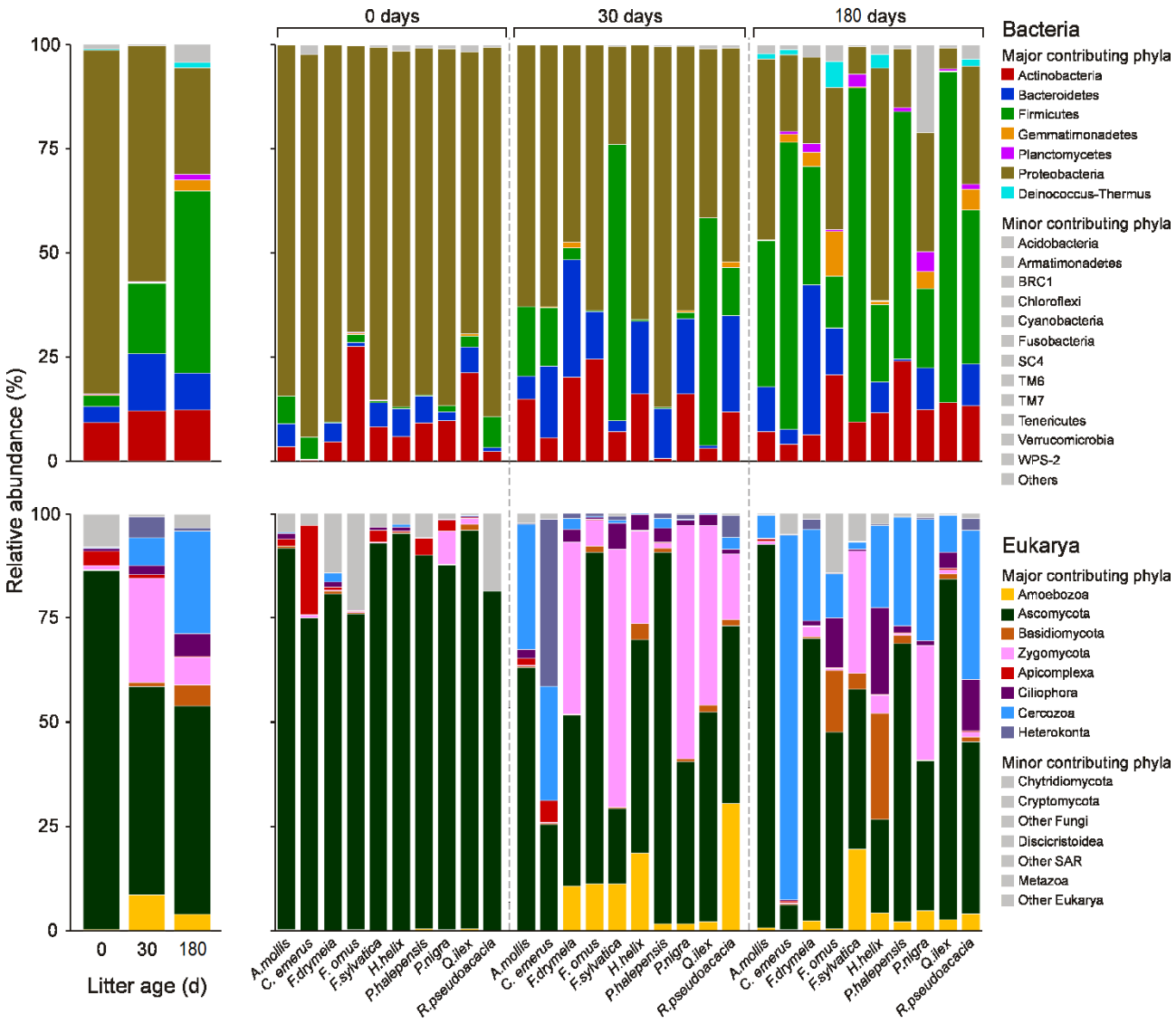

Figure 2.2 Relative abundance of bacterial (top) and eukaryotic (bottom) phyla in litter samples of different ages, either averaged (left) or separately reported for 10 different litter species (right).

Bacterial succession

Considering prokaryotic community composition at phylum level, *Proteobacteria* dominated on un-decomposed litter (mean ± standard error of relative abundance was 83.0±2.6%, Figure 2.2), followed by *Actinobacteria, Bacteroidetes* and *Firmicutes* (9.2±2.7%, 3.9±0.8% and 2.7±0.8%). As decomposition was proceeding, *Proteobacteria* were largely substituted by *Firmicutes*. *Actinobacteria* and *Bacteroidetes* generally increased with litter age, but the response was litter species-specific. For instance, *Bacteroidetes* thrived in 180-days old litters of *F. drymeia* (relative abundance 36.0%), but were not found in *F. sylvatica* materials. *Firmicutes* dominated in aged litter of *F. sylvatica* (80.1%), *Q. ilex* (79.4%) and *C. emerus* (68.9%) but were relatively rare in *F. ornus* (12.5%) and *H. helix* litter (18.5%). Minor contributing phyla, such as *Gemmatimonadetes, Chloroflexi, Planctomycetes* and others appeared almost exclusively in litter decomposed for 180 days, with mean relative abundance < 5% in most materials. *P. nigra* litter was an exception, showing a high

relative abundance of these phyla (overall 21.4%), whose major contributors were *Chloroflexi* (5.7%), mostly absent elsewhere.

Considering the most abundant genera (i.e. relative abundance >1% in at least one litter sample) the majority showed a significant shift in relative abundance during the decomposition process. For instance, members of *Sphingomonas* and *Pseudomonas* became progressively rarer during decomposition, being replaced by *Streptomyces*, *Bacillus* and *Paenibacillus* that showed the opposite pattern (Figure 2.3). In this regard, both NMDS (Figure 2.7A) and hierarchical Ward clustering (Figure 2.4) based on microbial composition at genera and family levels, respectively, showed a clear separation of the samples according to litter age.

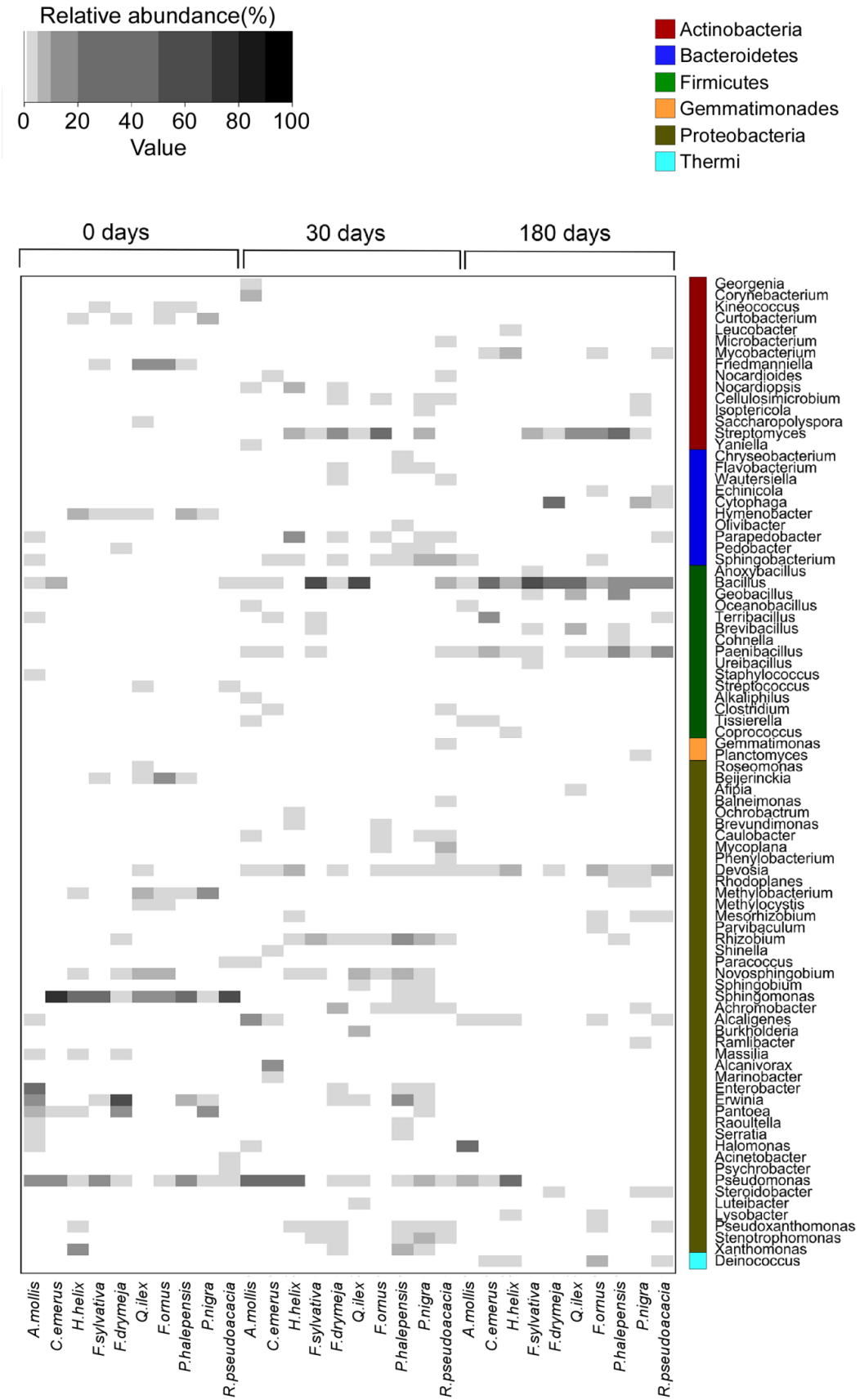

Figure 2.3 Relative abundance of bacterial genera in 30 litter materials (10 species at 3 different ages). Different colors indicate different phyla.

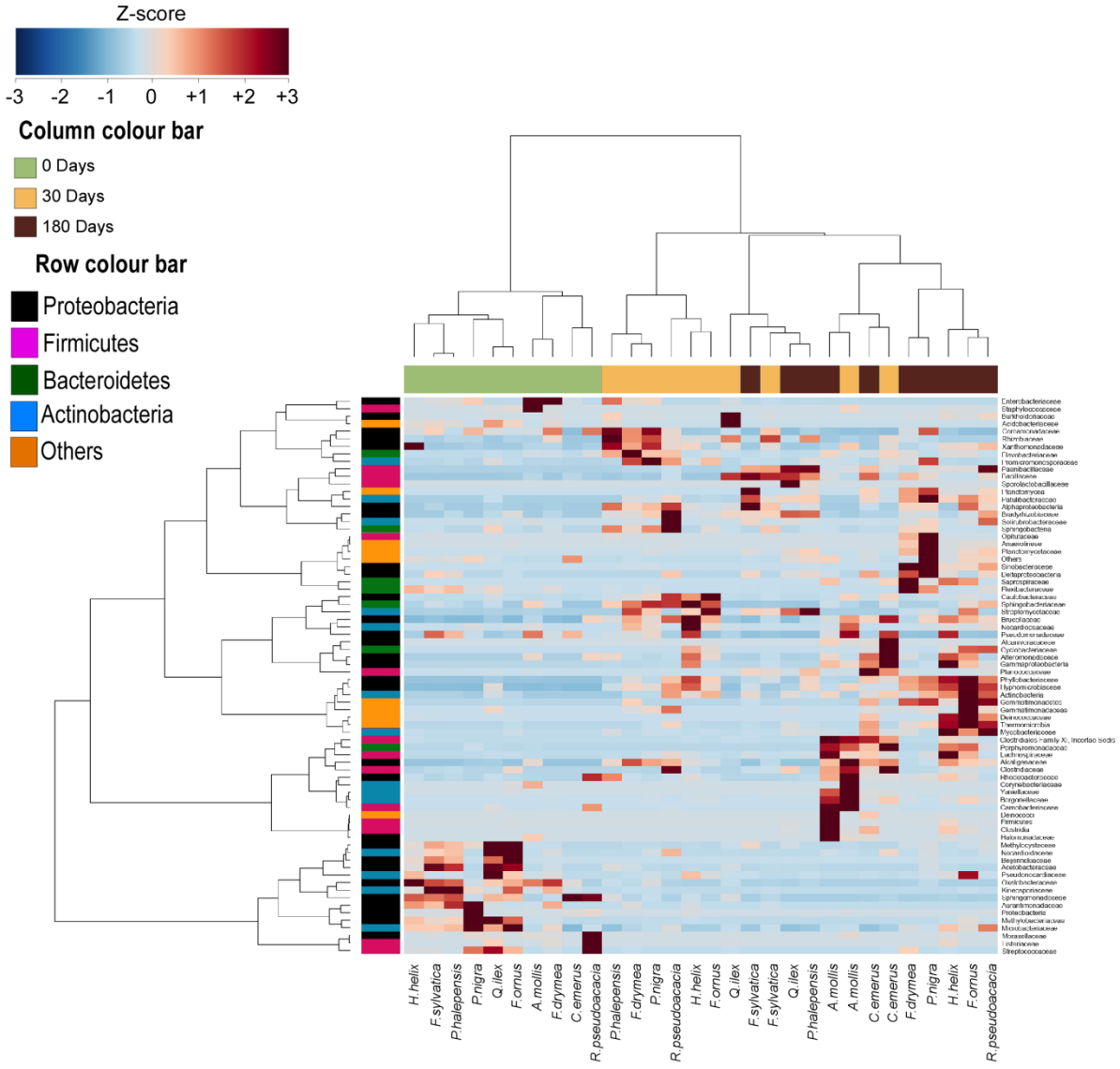

Figure 2.4 In the heatplot, hierarchical Ward-linkage clustering is based on the Spearman's correlation coefficient of the bacterial taxa abundance. The colour scale represents the standardized abundance of each variable, denoted as Z-score, with red indicating high abundance and blue indicating low abundance.

Eukarya succession

Members of the phylum Ascomycota dominated the eukaryotic community of all undecomposed litter materials (mean ± standard error of relative abundance was 86.6±2.5%, Figure 2.2). In some litter samples, however, other Eukarya were relatively abundant, as in the cases of protozoans belonging to the *Apicomplexa* in *C. emerus* litter (21.4%) and members of *Zygomycota* in *P. nigra* litter (7.8%). During decomposition, Ascomycota abundance rapidly decreased after 30 days (50.0±7.0%), remaining constant up to 180 days (49.9±8.5%). Members of the polyphyletic phylum Zygomycota were rare in fresh litter (1.0±0.7%), but underwent a dramatic increase after 30 days of decomposition (24.9±7.6%) especially in *F. sylvatica* (62.1%), *P. nigra* (56.2%), *Q. ilex* (43.5%) and *F. drymejia* (41.2%), litters. After 180 days, *Zygomycota* were still relatively abundant only in *F. sylvatica* (29.1%) and *P. nigra* (27.2%) litters, while being much rarer (1.4±0.4%) in all other materials. Members of *Amoebozoan, Basidiomycota* and *Cercozoa* were substantially absent in

fresh litter (0.1±0.1%, 0.2±0.1% and 0.3±0.2%, respectively), but more abundant in aged litters. Amoebozoan peaked in 30-days old materials (8.7±3.1%), then decreasing later on (4.0±1.8%), while Basidiomycota were progressively more abundant in 30- (1.0±0.3%) and 180-days (5.0±2.6%) old litters, showing highest abundance in aged litter of *H. helix* (25.6%) and *F. ornus* (14.9%). Such trend was even more evident for *Cercozoa* (6.8±3.7% and 24.6±7.8% in 30- and 180-days old litters, respectively), which became the dominant group in aged litter of the nitrogen-fixing species *C. emerus* (87.7%) and *R. pseudoacacia* (35.7%).

Most of the abundant Eukarya genera (i.e. relative abundance >1% in at least one litter sample), similarly to what observed for Bacteria, showed a shift of relative abundance during decomposition (Figure 2.5). Among Fungi, members of *Aureobasidium* were the dominant group in fresh litter, while at intermediate decomposition stage (30 days) *Rhizopus* and *Rhizomucor*, two members of the Zygomycota phylum, showed the highest relative abundance. At the latest stage (180 days) fungi belonging to *Aspergillus* and *Coprinopsis*, two genera of Ascomycota and Basidiomycota, respectively, were the most abundant. NMDS (Figure 2.7B) and hierarchical and Ward clustering (Figure 2.6), based on Eukarya composition at genus and family levels, respectively, clearly showed the importance of decomposition time in shaping the microbial community.

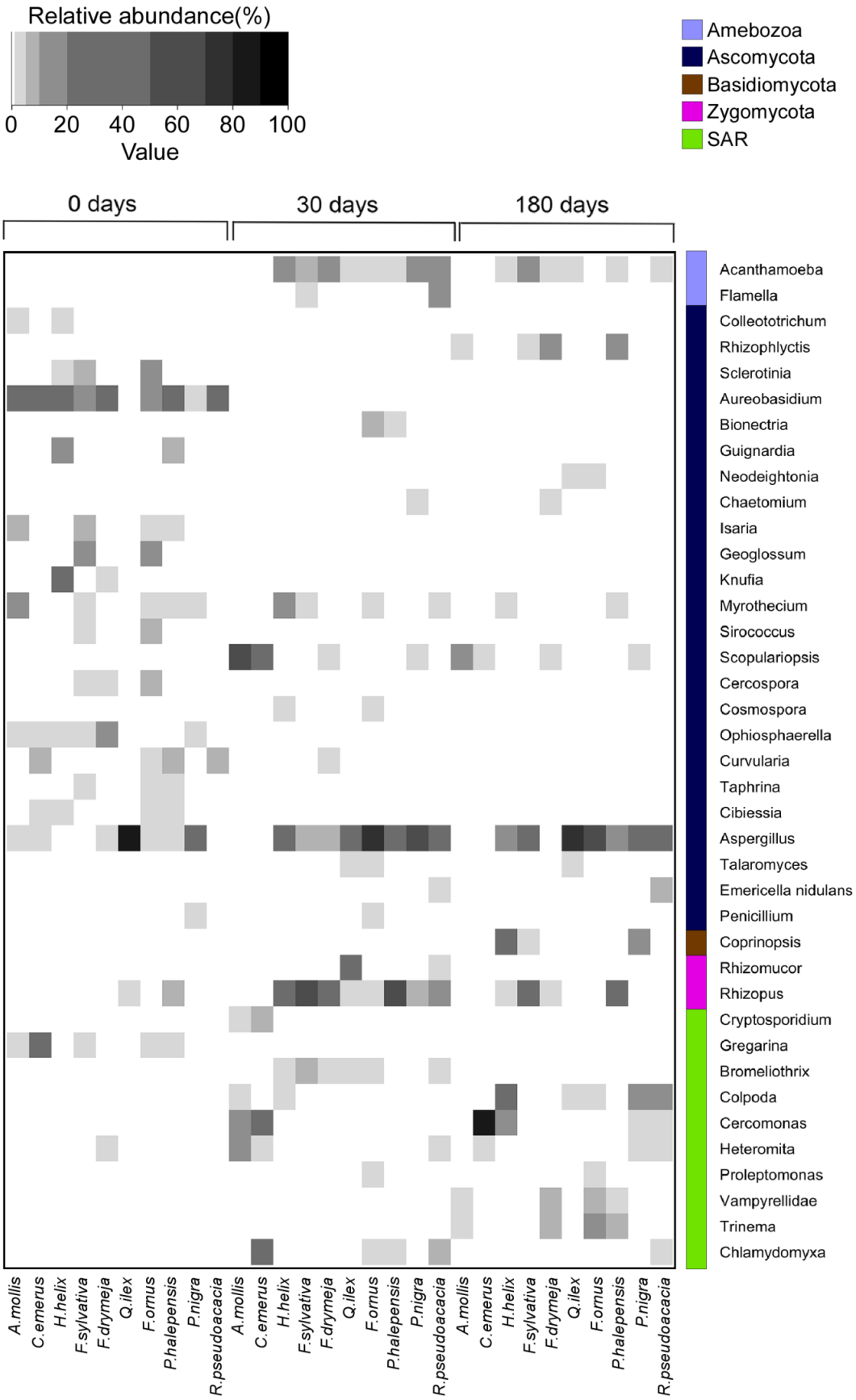

Figure 2.5 Relative abundance of eukaryotic genera in 30 litter materials (10 species at 3 different ages). Different colors indicate different phyla.

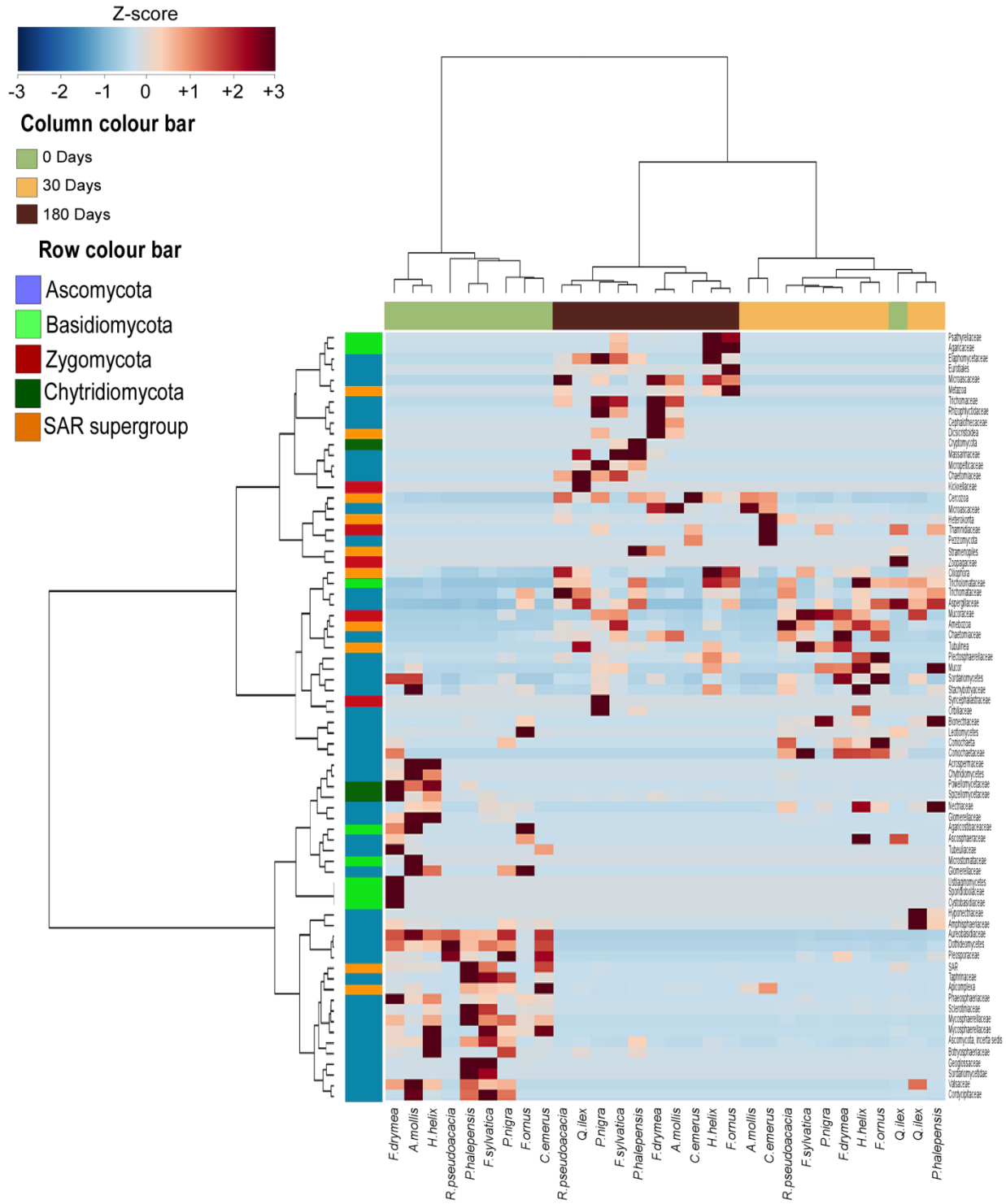

Figure 2.6 In the heatplot, hierarchical Ward-linkage clustering is based on the Spearman's correlation coefficient of the Eukaryotic taxa abundance. The colour scale represents the standardized abundance of each variable, denoted as Z-score, with red indicating high abundance and blue indicating low abundance.

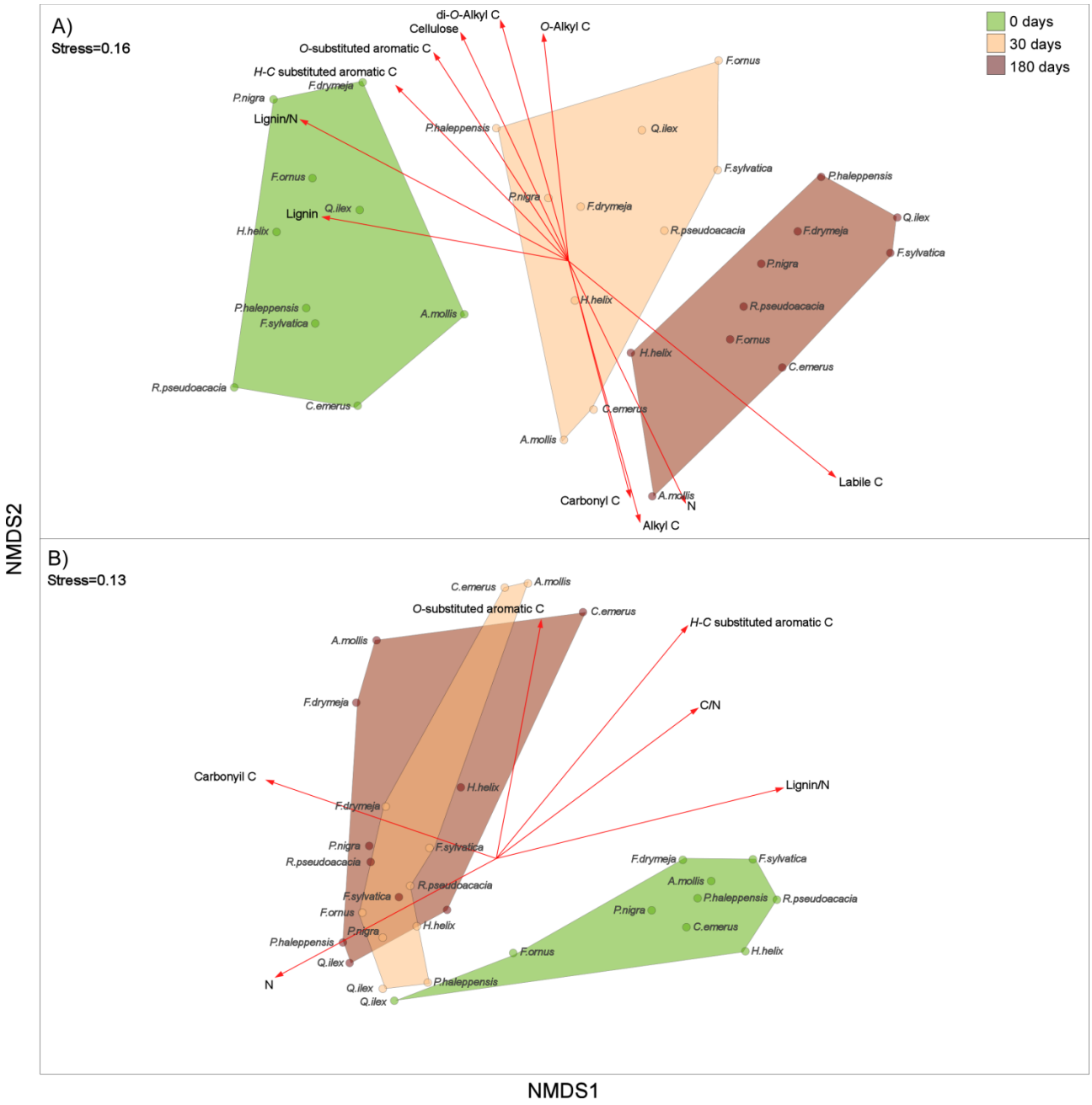

Figure 2.7: Results of non-metric multidimensional scaling (NMDS) of 30 litter materials (10 species at three different decomposition stage) based on the relative abundance of bacterial (A) and eukaryotic (B) phyla found in the samples. Litter quality parameters were also plotted, based on vector loadings.

C/N and lignin/N ratios largely varied among undecomposed litter species. Expectedly, C/N ratio significantly decreased with litter age for all materials (ANOVA; $P < 0.05$), with the exception of *A. mollis* (Figure 2.8), while lignin/N ratio consistently increased in all litter species. Litter N and lignin concentration changed during decomposition and in relation to the plant species, but with a general increase with litter age. Differently, cellulose content generally decreased with litter age, with higher rates in the cases of *F. drymeia*, *H. helix*, and *Q. ilex*.

^{13}C NMR spectra revealed significant and consistent differences among litter species, as well as changes in the relative abundance of different C types during decomposition (Figure 2.8). Fresh litter chemistry largely varied among species, with relative abundance of *O*-alkyl-C and di-*O*-alkyl-C fractions, which largely

correspond to sugars and polysaccharides, decreasing during the first 30 days of decomposition. On the other hand, the relative content of aliphatic alkyl-C, characteristic of lipid waxes, cutins and microbial by-products, increased during the first 30 days, particularly in litters of *A. mollis*, *C. emerus*, *F. drymeia*, *H. helix*, and *P. nigra,* then showing at most a slight increase at later stage. The relative abundance of aromatic C did not substantially change throughout the observation period, with the exception of *H*- and *C*-substituted C that showed a slight, but significant increase in materials decomposed for 180 days. Such late increase was also observed for methoxyl C. Finally, the relative content of the carbonyl C fraction did not significantly vary with litter age, showing limited changes of different sign in different materials.

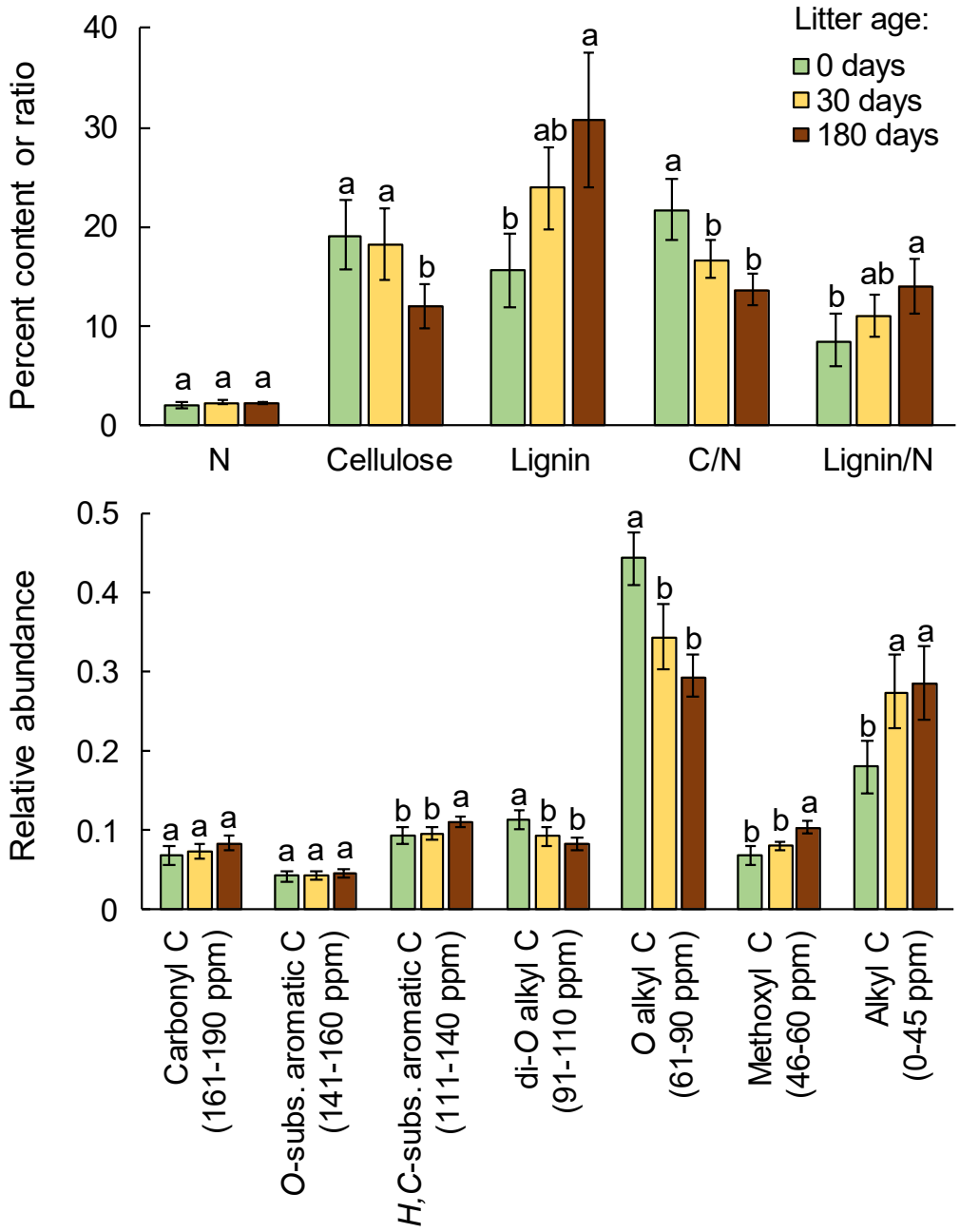

Figure 2.8. Dynamics of litter nitrogen (N), cellulose and lignin percent content, C/N and lignin/N ratios (top), and C-types corresponding to ^{13}C CPMAS NMR regions (bottom) during 180 days of decomposition. Data refer to mean of 10 litter species ±SD. Different letters within each chemical descriptor indicate age-dependent significant differences (Duncan test from one-way ANOVA at $P < 0.05$).

Linking microbial succession to litter chemistry

Correlation networks between litter microbiota and chemical traits provided a synthetic picture of the relationships occurring between litter microbial community composition, as related to litter type and age, and dynamic changes of litter chemistry during the decomposition process (Figure 2.9). In undecomposed materials, the large predominance of *Proteobacteria* was positively associated with the substrate C/N ratio, cellulose content, relative fraction of *O*-alkyl C and, to a lesser extent, Alkyl C (Figure 2.9A). *Proteobacteria* abundance was also negatively associated with litter N content, carbonyl and methoxyl C fractions (Figure 2.9B). A similar pattern of association was found for *Actinobacteria*, although with no link to Alkyl C, while the abundance of *Bacteroidetes* was positively related with the cellulose content and negatively with the labile C content and the carbonyl C fraction. *Firmicutes* showed an opposite pattern, with positive associations with litter N content and methoxyl C fraction, and negative correlation with the cellulose content and C/N ratio (Figure 2.9A and B). In the same undecomposed materials, correlation networks for Eukarya showed less consistent association patterns. The ubiquitous dominance of *Ascomycota* included genera positively associated with lignin/N ratio and carbonyl C fraction, and others negatively correlated with C/N ratio, labile C content, and methoxyl C fractions. Associations of *Ascomycota* with lignin content and *O*-alkyl C fraction included both positive and negative cases, likely indicating different feeding preferences within this phylum. Other cases of significant associations included *Cercozoa*, positively correlated with litter H,C- aromatic C fractions, and Zygomycota and Amoebozoa, negatively related with litter Alkyl C fraction and N content, respectively. Interestingly, genera from Apicomplexa, relatively abundant in undecomposed litter, were not associated with litter chemical traits considered in our analysis. The number of significant associations between litter microbiota and chemical traits dramatically increased after 30-days of decomposition (Figure 2.9C and D). The number of genera correlated with specific litter traits (i.e. edge linewidth in Figure 2.9) increased following the general increase of bacterial and eukaryotic diversity (i.e. node size in Figure 2.9). But more interestingly, the number of litter chemistry parameters associated to microbiota (i.e. number of nodes in Figure 2.9) increased as well. In particular, the increased number of taxa within all bacterial phyla, as well as the appearance of SAR super-group among Eukarya, not only reinforced the connections described above for undecomposed materials, but also corresponded to new relationships, as those with litter di-*O*-alkyl C and *O*-aromatic C fractions. Finally, after 180 days of decomposition, correlation networks generally showed fewer significant associations compared to less decomposed materials (Figure 2.9 E and F), indicating a lower litter chemistry-dependence of microbial community composition, and hence a higher compositional stability of bacterial and eukaryotic assemblages, compared to the preceding successional stages.

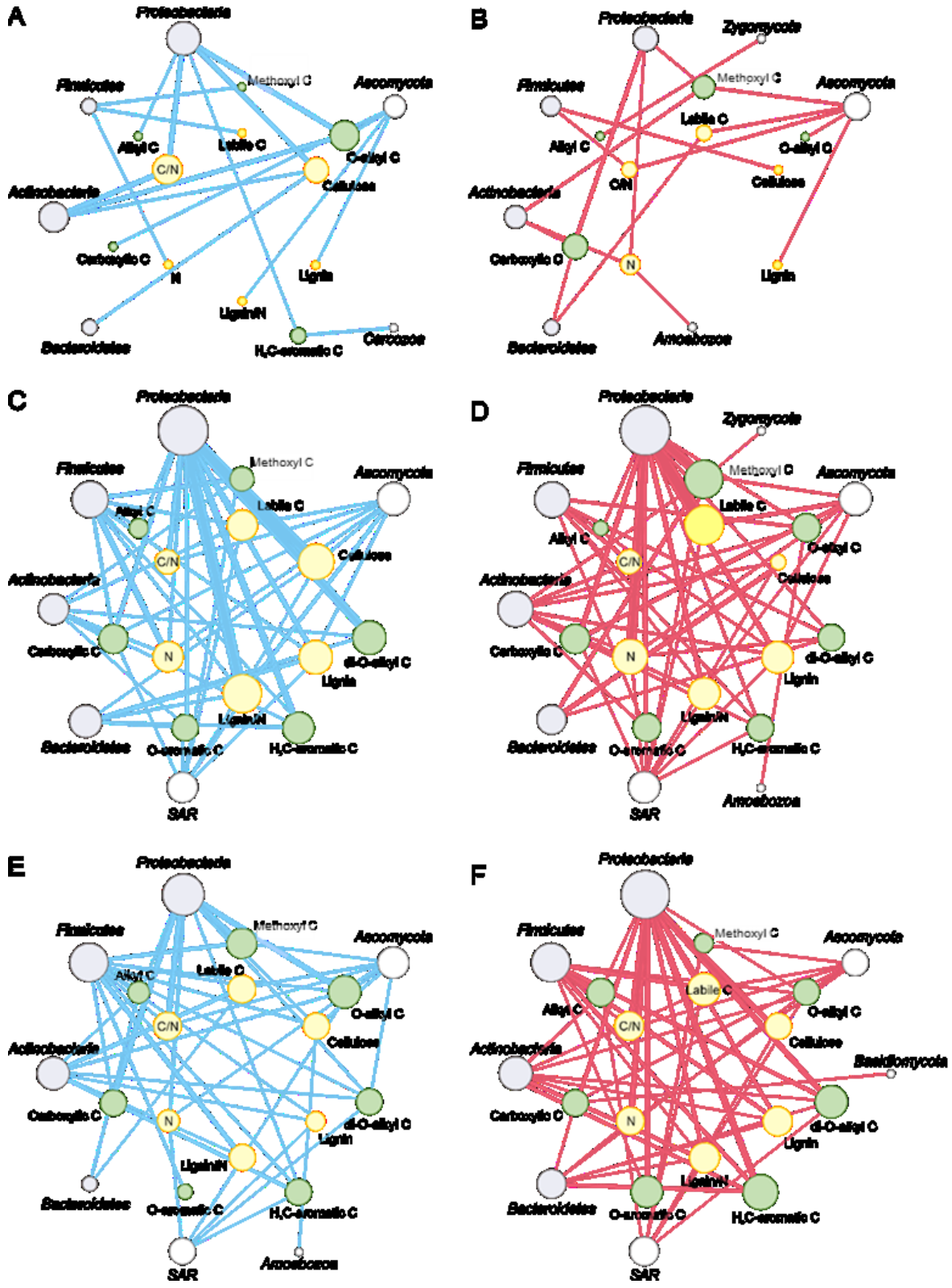

Figure 2.9 Relationship between microbiome and litter chemistry for 10 different litter materials either undecomposed (A-B) or after 30 (C-D) and 120 (E-F) days of decomposition. In each panel, data refer to correlation network between Bacteria and Eukarya phyla (grey and white nodes, respectively) and litter chemical traits (green and yellow nodes for ^{13}C NMR-based C types and elemental/proximate parameters, respectively). Node size is log-proportional to the total number of significant correlations observed within each phylum or litter trait. Linewidth is proportional to the number of OTUs in a given phylum significantly associated to a specific litter trait, either positively (blue lines, A, C, F) or negatively (red lines, B, D, E). Phyla with no OTUs associated to litter chemistry or litter traits unrelated to litter microbiome are not shown.

2.4. Discussion

Bacterial and eukarya diversity

Overall, we found a general increase in bacterial and eukaryotic taxonomical richness and diversity during decomposition, with the most relevant step corresponding to the early litter decay phase and a slight, but further increase at later stages. Similar results were reported by other culture-independent studies, showing a higher bacterial and fungal diversity in aged compared to fresh litter (Voříšková & Baldrian, 2013; Purahong *et al.*, 2016; Tláskal *et al.*, 2016). The progressive increase in microbial diversity over decomposition time is commonly explained with the rapid replacement of part of the phyllospheric community by more competitive saprotrophic bacteria and fungi. Thereafter, different highly diverse saprotrophic communities can co-exist as a consequence of the trophic specialization that follows the increase in litter chemical diversity occurring during decomposition, coupled to structural heterogeneity and the formation of microhabitats within the litter layer (Gartner & Cardon, 2004).

Bacterial successional dynamics

At phylum level, our multispecies experiment revealed similar successional trajectories among Bacteria. Firstly, we found that Proteobacteria dominated all undecomposed litter. Members of *Sphingomonas*, which dominated most of undecomposed litter types, were previously reported as constituents of phyllospheric communities (Purahong et al. 2016), hence presumably occurring in plant leaves prior to senescence. Similar considerations can be drawn for members of *Massilia*, *Methylobacterium*, *Pantoea,* some *Pseudomonas*, as well as the free-living nitrogen fixing *Beijerinckia,* that were reported in undecomposed litter as endophytes or epiphytes (Ruinen, 1956; Kutschera, 2007). Most of these genera rapidly disappeared during the early litter decay phase, and only *Pseudomonas* persisted at relatively high abundance after 30 days of decomposition, which is consistent with some studies reporting that members of this genus can use relatively complex organic substrates (Natsch *et al.*, 1994; Tomasi *et al.*, 1996). In general, most of the cultivable members of Proteobacteria phylum are classified as copiotrophs, i.e. fast-growing species that thrive in conditions of high resource availability. Many studies consistently reported a decrease in copiotrophic species during the early stage of litter decomposition, a pattern associated with the depletion of labile organic fractions (Aneja *et al.*, 2006; Keiblinger *et al.*, 2012; Urbanová *et al.*, 2015). In agreement with these observations, the relative abundance of Proteobacteria dramatically decreased during decomposition, a reduction that mirrors the rapid decrease in *O*-alkyl and di-*O*-alkyl NMR fractions associated with sugars and cellulose. Noteworthy, Proteobacteria almost disappeared in aged, N-poor and aromatic C-rich litter types, such as those of *Q. ilex* and *F. sylvatica*.

At intermediate and late decomposition stages, members of *Actinobacteria, Bacteroidetes*, and especially *Firmicutes* became progressively more abundant. *Actinobacteria* are known as degraders of highly recalcitrant organic materials, including xenobiotic and agrochemicals (Schrijver & Mot, 1999). Within this phylum, *Streptomyces* was the most abundant genus after 180 days of decomposition. Members of this genus are well-recognized as capable to degrade a variety of recalcitrant substrates, including biopolymers, fats, and aromatic compounds (Madigan *et al.*, 2017). Consistently, *Streptomyces* was especially abundant in aged litter of *F. sylvatica*, *P. halepensis* and *Q. ilex*, rich in aromatic C fractions. Moreover, *Streptomyces* members were

not found in litter of nitrogen-fixing species (i.e. *C. emerus* and *R. pseudoacacia*), regardless of their content in aromatic fractions.

After 180 days of decomposition, Firmicutes were the most abundant phylum in five out of ten litter types, highly prevailing in *C. emerus*, *F. sylvatica*, *P. halepensis* and *Q. ilex* litters. In general, members of this phylum are considered resistant to fluctuations in resource availability and environmental conditions, including desiccation (Battistuzzi & Hedges, 2009). Interestingly, for aged litters, we observed a negative correlation between the relative abundance of *Firmicutes* and the substrate C/N ratio, *O*-alkyl and di-*O*-alkyl fractions, and a positive association with the relative content of C that resonate within the 46-60 ppm spectral region. Such spectral region is commonly associated to methoxyl C, characteristic of lignin (Incerti *et al.*, 2017), but it can also include overlapping signals of *N*-alkyl C, as those from aminoacids and polypeptides (Bonanomi *et al.*, 2011). Within *Firmicutes*, *Bacillus* and *Paenibacillus* were the most abundant genera in all aged litter types. Members of these two genera share common trophic strategies, being able to degrade complex polymers by hydrolytic exoenzymes. Moreover, some *Paenibacillus* and *Bacillus* species can also degrade chitin, included fungal mycelium (Pleban *et al.*, 1997; Singh, PP *et al.*, 1999). In this regard, our results suggest that at late decomposition and successional stage, some members of *Firmicutes*, likely belonging to *Paenibacillus* and *Bacillus* genera, may feed over mycelial by-products from early colonizing Fungi.

In a recent study, reporting the occurrence of several nitrogen-fixing bacteria, including *Frankia*, *Rhizobium* and *Bradyrhizobium*, over decomposed *F. sylvatica* litter, Purahong et al. (2016) suggested that such functional guild might contribute to increase N availability during the decomposition process, hence promoting the activity of other microbes. Here, we found that nitrogen-fixing bacteria occurred both in undecomposed and 30-days aged litters. Free-living nitrogen-fixing *Beijerinckia* were exclusively present in fresh litter, disappearing later on. Differently, *Rhizobium* was rare in fresh and 180-days aged litters, but widespread in all 30 days old materials, with the exceptions of those showing the highest N concentration (*A. mollis* and *C. emerus*). On the other hand, *Rhizobium* was particularly abundant in litter species with low N content, such as *P. halepensis*, where it also exclusively persisted after 180 days of decomposition. These results suggest that nitrogen-fixing bacteria have optimal fitness for early-decomposed litter, and are excluded from ecological niches with very rich N plant tissues. Further studies are needed to assess if *Rhizobium* activity might alleviate microbial N starvation and, then, promote the decomposition of N-poor litter (Bonanomi *et al.*, 2017a).

Eukarya successional dynamics

Among Eukarya, fungi belonging to the Ascomycota phylum were dominant in undecomposed litter. In detail, *Aureobasidium*, a typical phyllospheric genus (Andrews *et al.*, 2002), was the most abundant on all fresh litter types, with the exception of *Q. ilex*, where it was replaced by *Aspergillus*, suggesting a competitive exclusion between these two taxa. However, *Aureobasidium* did not occur after 30 days of decomposition, being replaced by Zygomycetes and other ascomycetous fungi. According to previous culture-based studies carried-out on different trees species, the persistence of *Aureobasidium* and other phyllospheric fungi in decomposed litter is uncommon because of their limited competitive capabilities (Osono, 2002; Jumpponen & Jones, 2009; Voříšková & Baldrian, 2013). Differently, Zygomycota were rare in fresh litter but then showed

a peak of abundance after 30 days of decomposition, with *Rhizomucor* and *Rhizophus* being the most abundant genera. This pattern is consistent with the classic successional theory (Hudson, 1968; Frankland, 1998), predicting that these fast growing, opportunistic fungi are abundant limited to the early phase of decomposition, when the labile C fraction is still available in decaying litter. Consequently, this functional guild has often been referred as "sugar fungi" because of their marked preference for substrates rich in simple carbohydrates (Hudson, 1968; Osono & Takeda, 2001b). Accordingly, we observed an abrupt decrease of "sugar fungi" abundance after 180 days of decomposition, corresponding to the depletion of the litter *O*- and di-*O*-alkyl C fractions. Expectedly, "sugar fungi" were replaced by cellulolytic and ligninolytic fungi. However, some litter species-specific responses were observed, as in the case of *Rhizophus*, which was still relatively abundant after 180 days in *F. sylvatica* and *P. nigra* litters, or *Aspergillus* and *Zygomycota*, which rapidly disappeared from the labile C- and N-richest litters of *C. emerus* and *A. mollis*. Strong competition among "sugar fungi" and bacteria in these nutrient-rich and fast-decomposing substrates could be a possible explanation for these results.

Aged litters were dominated by *Aspergillus* and, limited to some cases of slow- (i.e. *F. drymeia* and *P. nigra*) and fast-decomposing litter (i.e. *H. helix*) with low relative content of the carbonyl C fraction, characterized by the occurrence of *Basydiomycota*, in particular the genus *Coprinopsis.* It is known that *Basydiomycota* with ligninolytic activity are characteristic of late stages of litter decomposition, as described in different previous reports e.g (Voříšková & Baldrian, 2013; Purahong *et al.*, 2016). In this respect, our study, being limited to an observation period of six months, cannot capture the complete fungal succession as related to litter chemistry dynamics at long term, suggesting the need for prolonged, longitudinal, multispecies experiments to reveal the link between specific litter molecular traits and the occurrence and persistence of climax microbial communities.

Our analysis, addressing the entire eukaryotic community, highlights a previously unnoticed but widespread occurrence of protists, mainly belonging to the *Amebozoa* and *Cercozoa* groups, in decomposed litter. Protists play a crucial role in soil, affecting plant growth and architecture, as well as the carbon and nitrogen cycles (Bonkowski, 2004). Acting as bacterial grazers, protists can shape the soil bacterial communities (Rønn *et al.*, 2002; Rosenberg *et al.*, 2009). Most current knowledge of protistan communities derives from soil studies (Gast *et al.*, 1996; Geisen *et al.*, 2014) et al. 2014), with a lack of information on decomposing litter. Our study showed that *Acanthamoeba* and *Cercozoa* protists are absent in fresh leaves, but widely distributed in aged litter. Moreover, protistan communities are non-randomly structured, showing strikingly distinct composition in relation to litter chemistry. In general, the peak in protist abundance observed after 30 days of decomposition is probably due to the trophic availability of bacterial prey, but such pattern presented specific exceptions. For instance, *Acanthamoeba*, a genus with few described species, but widespread in soil samples (Fiore-Donno *et al.*, 2016), was abundant in all litters, but not found in the labile C- and N-richest *A. mollis* and *C. emerus* materials, where it was completely replaced by *Cercozoa*. The reasons and processes underlying such species-specific association between protists and litter are unknown, but it could well be hypothesized an indirect effect of litter molecular quality, directly affecting the quality of the bacterial food source. More in general, the widespread presence and the relatively high abundance of protists in all litter types suggest that

such microbes could play an important role in shaping litter bacterial community, consistently to soil communities (Rønn *et al.*, 2002; Rosenberg *et al.*, 2009), in turn affecting the decomposition dynamics. However, further work is needed to clarify the trade-off between the top-down control of protists on bacterial community, and the bottom-up effect of litter molecular quality and the associated bacterial dynamics on protists community.

Linking litter chemistry to microbial succession

In general, it is well established that microbial decomposition affects litter chemistry and that such chemical changes further drive microbial turnover, hence controlling successional trajectories e.g. (Hättenschwiler *et al.*, 2005; Moorhead & Sinsabaugh, 2006; Chapman *et al.*, 2013). However, most previous studies could not unravel the specific association between litter molecular properties and microbiota, since based on either single litter types e.g. (Tláskal *et al.*, 2016), or broad descriptors of microbial communities, such as heterotrophic respiration or phospholipid fatty acid profiles (e.g. Baumann et al., 2009). Differently, in our study, most litters showed qualitatively similar but quantitatively different chemical shifts while ageing, producing specific effects on microbial communities. According to previous evidence (Preston *et al.*, 2009b; Bonanomi *et al.*, 2018b), besides the expected progressive depletion of labile C, our ^{13}C NMR analysis revealed the persistence of more stable molecular fractions, whose quality dynamically changed during decomposition, due to microbial transformation of pre-existing organic molecules into novel recalcitrant compounds, as well as selective preservation of the resistant fraction occurring into the original plant materials (Berg & McClaugherty, 2008; Preston *et al.*, 2009b). These processes, common to all substrates, proceeded with different rates in different litter species, (e.g. very rapid *A. mollis*, *C. emerus*, *H. helix* and slow for *F. sylvatica*, *P. halepensis*, *Q. ilex*), according to the broad range of litter molecular properties selected for this study. As such, using large and diverse sets of materials could help to highlight the interconnections between litter chemistry, and diversity and composition of the decomposing microbiota, as also recently reported by Bhatnagar (2018).

We found that the impact of litter chemistry on microbial community structure increased during the decomposition process, being of little importance in the case of freshly fallen leaves, but becoming the most controlling factor in aged litter. Indeed, our correlation network analysis identified only 28 significant associations between the microbial community and undecomposed litter chemistry parameters, suggesting that the biotic interactions play a major role in selecting bacterial and fungal endophytes (Bulgarelli *et al.*, 2012). In this context, C/N ratio, cellulose content and the carbonyl and *O*-alkyl C fractions of the ^{13}C NMR spectra appeared as the most important chemical parameters.

On the other hand, the number of significant associations identified by our network analysis dramatically increased to 95 and 94 for 30- and 180-days aged litters, respectively. In addition, we interestingly found a shift in the association direction (i.e. from positive to negative correlation or vice versa) for several chemical parameters passing from 30 to 180 days old litters, indicating a complex, dynamically changing interconnection between litter chemistry and microbiota composition. For instance, after 30 days of decomposition, members of Proteobacteria were generally positively and negatively associated to litter lignin/N ratio and carboxilyc C fraction, respectively, but such relationships shifted to negative and positive, respectively, after 180 days.

Considering the set of litter chemistry parameters considered in this study, proximate cellulose, labile C content and lignin/N ratio were those associated to most OTUs, while litter C/N ratio was apparently much less predictive of microbiota dynamics. This is in agreement with previous studies e.g. (Bonanomi *et al.*, 2013), in which C/N was criticized as unreliable descriptor of decomposed litter, as it only considers organic C quantity, but not its quality in terms of different molecular types and associated functions review in (Cartenì *et al.*, 2018). Our study provides a novel contribution to this general topic, extending the limitations for C/N use as functional index of litter chemistry, when the specific objective is to describe or predict the relationships between decomposed litter quality and decomposer community structure and composition.

Considering ^{13}C NMR parameters, *O*-alkyl, carbonyl and methoxyl C fractions were those associated to most OTUs at early decomposition stage, whereas at later stages aromatic, alkyl and di-*O*-alkyl C fractions played the utmost roles. For instance, decomposer microbiota in undecomposed litter was unrelated to the *O*-aromatic C fraction, while after 180 days of decomposition the litter content of such molecular type was negatively associated with several bacterial groups (i.e. Firmicutes, Bacteroidetes, and Proteobacteria), positively with Actinobacteria and protists, and unrelated to Fungi. The increasing frequency of association between aromatic compounds and litter microbiota as decomposition was proceeding is consistent with the progressive accumulation in litter of organic aromatic molecules (Berg & McClaugherty, 2008; Preston *et al.*, 2009b). In this respect, previous studies on different litter functions, in relation to molecular characterization by ^{13}C NMR, showed that dynamic variations of specific spectral signals can be highly predictive of decomposition rates (Bonanomi *et al.*, 2013), soil hydrophobicity (Cesarano *et al.*, 2016), and aggregation capability (Sarker *et al.*, 2018), particularly for already decomposed materials, thus capturing the influence of C molecular quality on soil functioning. This study confirms the usefulness of describing litter molecular quality by ^{13}C NMR, providing new insights towards the understanding of the complex link between litter quality and the associated microbiota.

2.5. Conclusions

Our study showed that leaf litter during decomposition undergoes significant chemical transformations with associated microbiological successional shift. Despite the wide chemical range of litter materials purposely selected for our decomposition experiment, most litter types remarkably shared similar molecular changes and successional trajectories. A such, our findings suggest a common pattern in the microbial community compositional response to the chemical changes that occur during decomposition. Within this general framework, we could also identify litter-specific successional patterns associated with specific chemical fingerprinting revealed by ^{13}C NMR analysis. In this regard, the combination of ^{13}C NMR analysis with high-throughput sequencing provides a new approach to reveal the complex interconnections between the substrate chemical changes and the microbial community shaping that co-occur during litter decomposition.

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3. Role of Ectomycorrhizal symbiosis in litter-mediated plant soil feedback

Overview

Monodominance is a particular phenomenon in plant communities. Normally the conditioning effect of plants on its own soil by effect of litter autotoxicity induce an increase of species richness. Most of ecosystems dominated by a single species are characterized by plants able to forms ectomycorrhizal symbiosis. Ectomycorrhizal monodominated stands occurs largely in boreal and temperate climatic belt and paradoxically occurs also as patches in tropical ecosystems. Has been demonstrated that ectomycorrhizal symbiosis prevents plant soil negative feedback by inducing increased ability for plants to obtain resources from the soil matrix and resistance to pathogen attacks. However, few works studied the relation between ectomycorrhizal symbiosis and litter autotoxicity as it could became positive for the plant carrying the symbiosis. Here we provide a bibliographic assessment of the state of the art on the knowledge about the monodominance in tropical forests, where the mitigation of the plant soil negative feedback by the symbiosis is stronger. Additionally, we carried out some experiments assessing in a full factorial design the relation between ectomycorrhizal symbiosis, litter age and presence respect plant growth assessed as plant biomass and structure of root apparatus. Moreover, the need of soil microbiota, besides the symbiont provided to plants, has been assessed. We observed by the bibliographic survey that the ectomycorrhizal symbiosis is quite common in tropical environment characterized by well-drained soil and is present in each continent with tropical climate. More particularly these communities when compared to common tropical forests has a great tendency to accumulate higher amount of conspecific litter in forest floor. This phenomenon is known as Gadgil effect in which ectomycorrhizal fungus competitively excludes soil saprotrophs in the litter decomposition process. The ability to degrade conspecific litter and obtain advantage for symbiotic conspecific plant bypassing the autotoxic effect of the litter is confirmed in the experimental sections. These results explain the importance of autotoxicity originated from litter as one of the main driver structuring plant communities and, moreover, suggest that plants developed ectomycorrhizal symbiosis as effect of the fitness induced by litter autotoxicity.

3.1. A review on the rare case of monodominance in tropics.

3.1.1. Introduction

Tropics are the most important reservoir of plant biodiversity on the earth (Richards, 1952; Wilson *et al.*, 2012). In that regions, hundreds of plants species are able to coexist in a single hectare of forest rising in a hyper-diverse ecological context (Valencia *et al.*, 1994; Ter Steege *et al.*, 2003). Such plant biodiversity, attract the attention of several community ecologists with the intent to detangle the ecological processes shaping those natural assemblages.

Diverse theories were formulated in the last century trying to explain plant coexistence patterns in tropics (Wright, 1992; Wright, 2002; Leigh Jr *et al.*, 2004; Harrison, 2005; Schupp & Jordano, 2011). In light of this, a comprehensive review effort were achieved in 2002 by (Wright) that lists the 4 main ecological theories explaining species coexistence between plants in tropical ecosystems: (1) niche partitioning (each species are able to occupy a precise favourable position or exploit different nutrients sources avoiding niche overlap with other species); (2) niche differences associated with a trade-off between survivorship and growth; (3) Janzen-Connell effect (the decrease of recruitment near fruiting conspecifics by host-specific pests) or Plant soil negative feedback (instauration of negative condition for plant survival given biotic and abiotic changes in soil), and (4) negative density dependence among the more abundant species at larger spatial scales (Hypothesized by Whrite as sole theory not able to exclude the others). Despite the huge amount of studies focusing on coexistence of hyper-diverse tropical forests, few works centred the attention on monodominant forests in tropics (Figure 2.1.1 Scopus data source). In the heterogeneous distribution of plant species characterizing tropical regions, few but commonly recurrent, patches of canopy were detected as dominated by a single species (Whitmore, 1978). The monodominated forested patches were observed in different location of tropical belt with a variable coverage ranging from few to hundreds of hectares (Connell & Lowman, 1989). Focusing on this, (Hart *et al.*) in 1989 describes a series of case regarding old growth forests dominated by single species and additionally providing a comparative study between mixed and monodominated forest with *Gilbertodendron drewerii* as dominant species. Detailed information on stand structures, soil characteristics, seed dispersal and renewal were provided. Finally, Hart suggested monodominance in tropical forests is a process mediated by low frequency and scale disturbance promoting the competitive exclusion process in support of a single species respect the others and relating it to edaphic characteristics of well-drained soil. In the same year, (Connell & Lowman) reviewed the concepts of monodominant forests providing different hypotheses explaining the anomalous patches in hyper-diverse tropics. Above all, appeared that instauration and stability of a monodominant forests were correlated with the type of mycorrhizal association between dominant plants and ectomycorrhizal fungi. Indeed, monodominant species were almost ectomycorrhizas rather than mixed forest that were exclusively associated with Arbuscular mycorrhizas. For simplicity all the case of monodominant forests in tropics are listed in Table 3.1.1 including mycorrhizal type association state. Nevertheless, few exception of monodominant AM forests were listed as *Mora excelsa* and *Prioria copaifera* in Guyana shield (Torti *et al.*, 2001) and *Peltogyne gracilipes* in Brazil (Villela & Proctor, 2002). However, the exception listed above seems to be ascribed to waterlogged characteristics of the environment (swamp) that should be more

favourable to AM (Carvalho *et al.*, 2004) rather than EM association (Sumorok *et al.*, 2008) on *M. excelsa* and *P. copaifera* and to high concentration of toxic Mg in *P. gracilipes* litter . Nowadays, the theory supporting monodominant forests triggered by EM association is still an open debate given the idea that no more that EM association could cause low diversity forests since the former exclude factor as abiotic soil quality and disturbance (Lodge, 1989; Peh *et al.*, 2011).

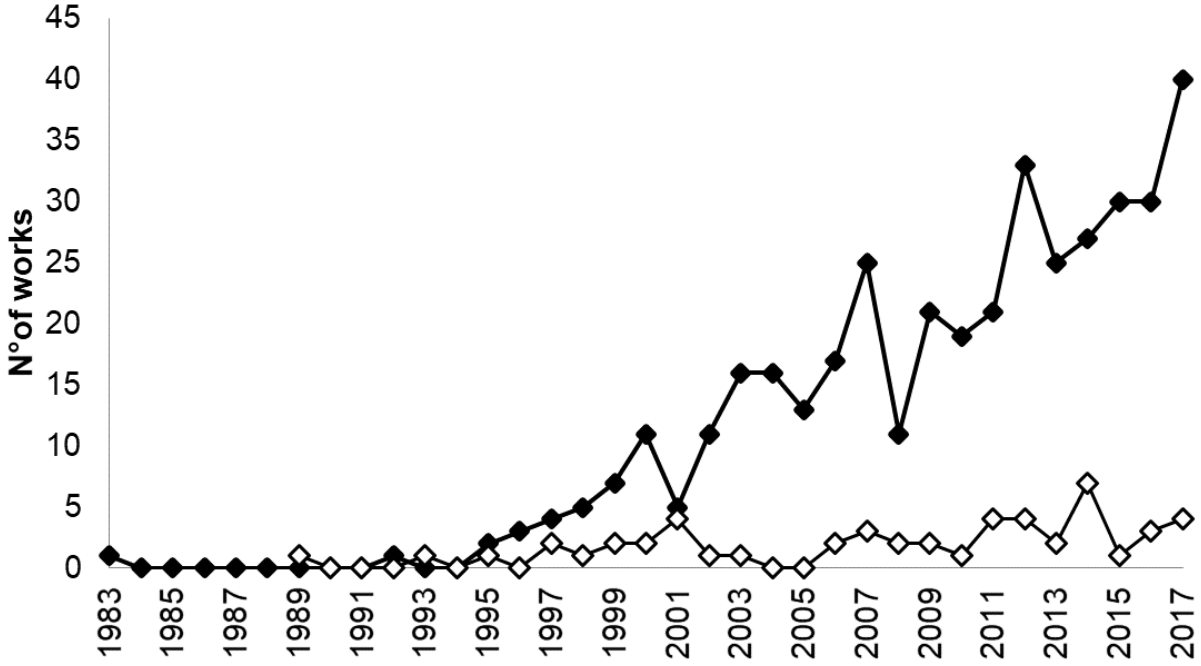

Figure 3.1.1 Trends of N° of works in time series (1983 to 2017) showing comparison of scientific production in community ecology between species richness (black squares) and monodominance (white squares) in tropical areas.

Table 3.1.1 list of scientific work on monodominance in tropical woodland ecosystems presents in literature. In table is specified location, plant species, plant family, dominance percentage in which works are focused. Additionally, symbiotic association with mycorrhizal fungi is reported when specified.

Location	Dominant species	Family	D°	EM	AM	References
Malaya	*Shorea curtisii*	*Dipterocarpaceae*	Nr	Y	?	Whitmore 1984;Burges 1969
Malaya, Sumatra	*Driobalanops aromatica*	*Dipterocarpaceae*	Nr	Y	Nr	Whitmore 1984;Lee 1967
Borneo,Sumatra	*Eusideroxylon zwageri*	*Lauracea*	Nr	?	Nr	Koopman&verhoef 1938
Trinidad	*Mora excelsa*	*Moraceae*	Nr	?	Y	Beard 1946; Torti 2002

East Africa	*Cynometra alexandri*	*Caesalpinoidae*	Nr	Y	Nr	Eggeling 1947
West Africa	*Talbotiella gentii*	*Caesalpinoidae*	Nr	Y	Nr	Swaine & Hall 1981;(Peh *et al.*, 2011) (literature within)
Central Africa	*Gilbertiodendron dewevrei*	*Caesalpinoidae*	60-90%	Y	Y	Gerard 1960 Louis 1947
Central Africa	*Julbernardia seretii*	*Caesalpinoidae*	Nr	Y	Nr	Gerard 1960
Peninsular India	*Tricalysia sphaerocarpa*	*rubiaceae*	72%	Nr	Nr	(Anbarashan & Parthasarathy, 2013)
Peninsular India	*Strychnos nux-vomica*	*loganiacee*	67%	Nr	Nr	(Anbarashan & Parthasarathy, 2013)
Peninsular India	*Dimorphocalyx glabellus*	*Euphorbiaceae*	60.8%	Nr	Nr	(Anbarashan & Parthasarathy, 2013)
Guyana	*Dycimbe corimbosa*	*Caesalpinoidae*	30-100%*	Y	Nr	Henkel 2001; Henkel 2003
Guyana	*Dicymbe altsonni/jenmanii*	*Caesalpinoidae*	70-75%*	Y	Nr	Henkel 2001; Isaac et al.1996
French Guiana	*Spirotropis longifolia*	*Papilionoideae*	Nr	No	Y	Henkel 2001; Isaac et al.1996
Brazil	*Peltogyne gracilipes*	*Caesalpinaceae*	86%	Nr	Nr	(Villela & Proctor, 2002)
New Caledonia	*Nothofagus aequilateralis*	*Notofagaceae*	80%	Y	Nr	(Read et al., 1995)
Guyana	*Mora excelsa*	*Moraceae*	Nr	No	Y	(Torti et al., 2001)
Guyana	*Prioria copaife*	*Caesalpinoidae*	Nr	No	Y	(Torti et al., 2001)
Central America	*Quercus oleoides*	*Fagaceae*	Up to 100%	Y	Nr	(Boucher, 1981)
Central Africa	*Aucoumea klaineana*	*Burseraceae*	Nr	?	Nr	(Peh et al., 2011)(literature within)

Central Africa	*Tetraberlinia tubmaniana*	*Caesalpinoidae*	Nr	Y	Nr	(Connell & Lowman, 1989; Peh et al., 2011)(literature within)
Malesia	*Parashorea malaanonan*	*Dipterocarpaceae*	Nr	Y	?	Richards 1996 (Peh et al., 2011)(literature within)
South america	*Aspidosperma excelsum*	*Apocynaceae*	Nr	?	Nr	Richards 1996(Peh et al., 2011)(literature within)
South america	*Dacryodes excelsa*	*Burseraceae*	Nr	?	Nr	Richards 1996(Peh et al., 2011)(literature within)
Central America	*Celaenodendron mexicanum*	*Euphorbiaceae*	Nr	?	Nr	Martijena 1998(Peh et al., 2011)(literature within)
Panama	*Oreomunnea mexicana*	*Junglandaceae*	Nr	Y	Nr	(Corrales et al., 2016)
South america	*Eperua falcata*	*Caesalpinoidae*	Nr	Y	Nr	(Torti et al., 2001)

3.1.2. Mycorrhizas in tropical regions

Interestingly, comparative studies between AM and EM mycorrhizzal association are abundant in literature (Brundrett, 1991; Read, 1991; Smith & Read, 2010). Although both kind of mycorrhizzas do no shows high differences in the extent to which enhanced nutrient uptake is promoted, are dissimilar the way in which AM and EM operates. AM is an invasive intracellular association promoting, through hyphae elongation in extraradical matrix, the uptake of available nutrients (Smith & Read, 2010). Inversely, EM association is an intercellular association (few cases of penetration in cytoplasmatic matrix of root cells) with a strong zymogenous activity able to degrade directly organic matter in soil with a process similar to white/brown-rot saprotroph fungi (Dittmann *et al.*, 2002; Read & Perez-Moreno, 2003; Baldrian, 2009). The different ability of EM association in nutrients uptake result in out-competition of saprotrophs, that are demonstrated to be less present in EM forests and also known as Gadlid effect (McGuire *et al.*, 2013; Talbot *et al.*, 2013). Furthermore, the difference in the ability to degrade directly organic matter in soil is suggested by the high accumulation of litter in EM with respect to AM forests (Henkel, 2003; Mayor & Henkel, 2006; Woolley *et al.*, 2008) i.e. In *Tetraberlinia* and *Microberlinia* groove forests in center of Africa thick mass of EM hyphae degrade the litter produced by the consociated plant species (Chuyong *et al.*, 2000). Besides, increased litter accumulation in tropical forests shows a common pattern with temperate forests where litter accumulation is mainly due to common spatial distribution of fungal community in forest soils (McGuire *et al.*, 2013). These evidences propose, in addition, a more conservative ability of EM to obtain nutrient from litter (Osono, 2007; Corrales *et al.*, 2016).

3.1.3. Ectomycorrhizal symbiosis and Plant-soil feedback

Interestingly in the last decades, plant soil feedback attracting the attention of plant ecologist as mere explanation of plant community assemblages all over the world. Plant-soil feedback is explained in the imposition by pre-existing plant of particular conditions in soils affecting positively or negatively recruitment and growth of renewal both directly (conspecific) and indirectly (heterospecific) (Van der Putten *et al.*, 2013). In tropical stands plant soil negative feedback effect match Janzen-Connell distribution where seedlings shows higher health and recruitment increasing the distance from mother plant (Schupp, 1992). As matter of fact, Janzen and Connell distribution produce gaps where recruitment is available for heterospecific resulting in hyper-diverse forests. Afterward, several mechanisms were ascribed to be responsible of negative condition for recruitment beneath mother plant (Cesarano *et al.*, 2017a): 1) Nutrient depletion in the niche partitioned on conspecifics (Petermann *et al.*, 2008); 2) Species specific soil-borne pathogens accumulation in proximity of mother plant (Packer & Clay, 2000); 3) Allelopathy by root exudation (Ehrenfeld *et al.*, 2005); 4) Involvement of extracellular self-DNA as species specific inhibitor biomolecule (Mazzoleni *et al.*, 2015a). In light of this, positive plant-soil feedback is the mitigation or the disappearance of such negative conditions amplifying the magnitude of occurrence of a plant species rising in monodominace (Ehrenfeld *et al.*, 2005; Van der Putten *et al.*, 2013) as in the case of some tropical forests. Positive feedback has been demonstrated in temperate forests in EM tree where seedlings survival where enhanced beneath fruiting conspecific, suggesting the action of EM fungi provide: enhanced nutrient uptake in throphic niches already occupied by conspecific, protection from pathogens and increased ability to degrade organic matter and their potential negative compounds((Bennett *et al.*, 2017);(Fukami *et al.*, 2017)). In tropical regions this assumption, and the hypothesis connected to it, is supported by the multitude of works providing structural data of monodominant stands and its renewal. Such surveys in monodominant forests assessed homologous growth behavior between seedlings, saplings and poles divided by age classes, showing to be insensitive by the distance from a fruiting conspecific (Henkel *et al.*, 2002; Henkel, 2003; Henkel *et al.*, 2005);(Gross *et al.*, 2000; McGuire, 2007). Furthermore, the lasts suggest Janzen and Connell distribution is ineffective in monodominant stands (Laliberté *et al.*, 2015). Auxiliary support is provided by observation in temperate *Abies Alba* forests in Taburno mountain where isolated fruiting plants of *Fagus sylvatica,* a well-known EM tree, do not shows Janzen & Connell distribution of renewal; or in *Fagus sylvatica* forests, in Pollino mountain, where seedling survival of a suspect AM tree the leguminous *Laburnum alpinum* shows opposite behaviour (personal communication).

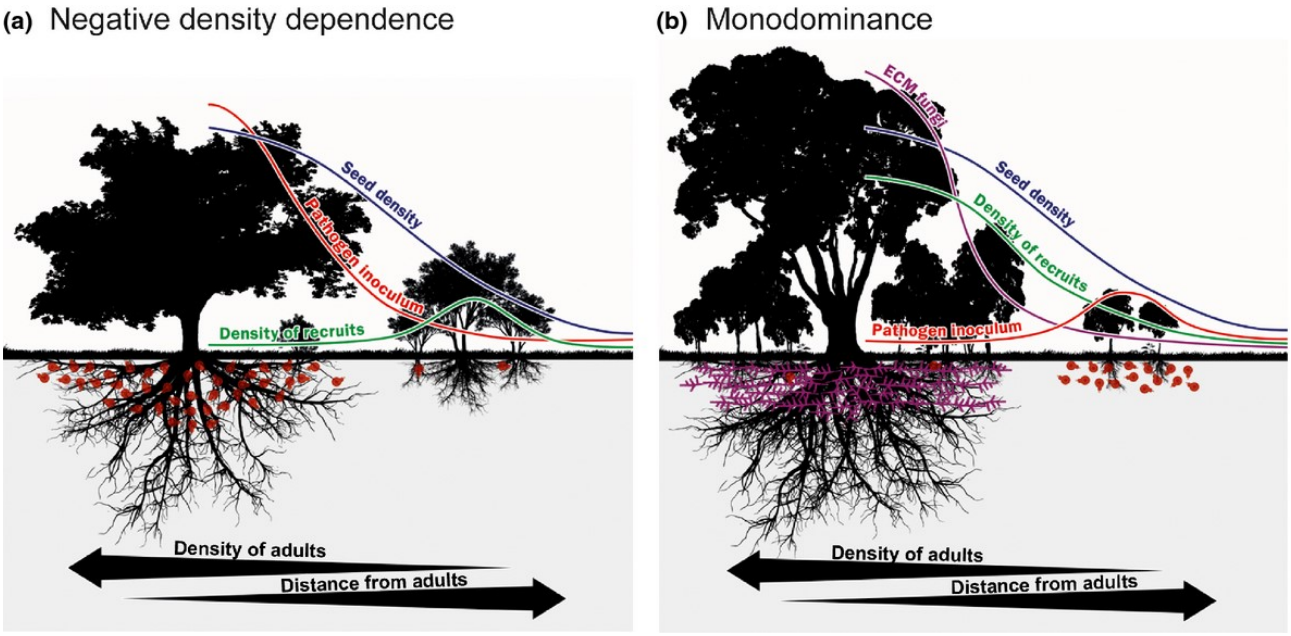

Figure 3.1.2 : Graphic representation of Density dependent distribution of renewal on non EM trees (a) and renewal distribution in monodominant EM forests (b from (Laliberté *et al.*, 2015)

3.1.4. Criticisms about the ectomycorrhizal dependent monodominant forests

Theory regarding Ectomycorrhizal association as factor promoting monodominant stand is still a matter of debate. Indeed, biases has been postulated on the concept as matter of fact that if EM association is so strong to outcompete other species, tropical forests should show homogeneous characteristics of canopy layer with a single species dominating the other; Possible explanations lay in the different environmental requirement and characteristics of EM associations. Indeed, heavy precipitation and not well drained soils limits spreading of EM tree as EM fungi are susceptible to Hypoxia (Hart *et al.*, 1989; Lodge, 1989; Read, 1991; Torti *et al.*, 2001; Carvalho *et al.*, 2004; Smith & Read, 2010; Peh *et al.*, 2014) in fact some works also reported monodominance occurrence in hill crests and well drained soils (Read *et al.*, 1995; Demenois *et al.*, 2017); High disruptive, rather than dispersive, action of predation of seeds, as in the case of *Quercus oleoides,* (Boucher, 1981); Besides, EM association is costly for plant host, plants support the growth of extended hyphal network with strong enzymatic production and uphold production of high masses of carpophores providing photosynthates (around 40% of production of Photosynthesis)(Lindahl *et al.*, 2005; Osono, 2007; Smith & Read, 2010; Talbot & Treseder, 2010) and receiving enhanced nutritive advantage, defense against pathogen and drought stress and the opportunity to exchange nutrients and information with conspecific through CMN (Common (Ecto)-mycorrhizal Network)(McGuire, 2007; Ebenye *et al.*, 2017). In light of those finely regulated but costly processes, and the functional feature of EM, is plausible to suppose that monodominance is not widespread everywhere in tropical regions. Further bias is proposed by the fact that ectomycorrhizal symbiosis is more common in Pinaceae trees that are rare or absent in tropical belt (Turner & Cernusak, 2011) and do not form monodominant stands in exception of few cases in extreme environmental conditions as boreal and temperate alpine environments (Fragniere *et al.*, 2015). Furthermore, Pinaceae are exclusively EM symbionts

in adult stages (Brundrett, 1991) rather than, in most case, in last years has been demonstrated the ability of monodominant plant previously considered exclusively EM to form AM associations i.e. Fagaceae; Dipterocarpaceae; Caesalpiniaceae. The last assumption explains that the combination of both mycorrhizal types, with prevalence towards EM, could be the main but not the sole factor promoting monodominance. As a matter of fact, in table 1 is possible to evaluate the prevalence but not the dominance of EM consociations as absolute factor to achieve monodominance. This should be given to particular condition related to the instauration of positive feedback as well as explained by (Brookshire & Thomas)in 2013 that centralize the attention to nutrient availability and suppose particular ability for N storage in *Mora sp*. forests. The lasts observation should be helpful in suggest the way in which monodominance and hence Plant soil feedback should be linked to nutritional strategies of woody plants both in EM and AM association.

3.1.5. Concluding remarks

Further evidence and experimental effort are required to obtain a general overview of Monodominace in tropical regions. Plausibly, the truth could lay in the soil. From literature gathered regarding the topics, full information concerning abiotic and biotic feature of focal plants and stand structure of above ground portion is actually provided, while few information is present about soil biota and its functionality in tropical monodominant forests, although nutritional advantage provided to plants where well described for both ECM and AM.

In best of our knowledge, literature lack works able to put together detailed information in decomposition from the whole soil microbiome including saprotrophs, mycorrhizas and bacterial communities. Supplementary information should be integrated in the way in which EM trees affect its own nutrient cycle and estimate which EM fungus species majorly affect the process. Molecular techniques should be the way to understand part of the community present in the soil avoiding the problems rising from unculturable species or difficultly ones. Nevertheless, Sequencing techniques provide qualitative but no quantitative information and result to be poor in answering capability regarding who eat what. Furthermore, sequencing techniques of soil samples require heavy manipulation with possibility of occurrence of sample alteration. Further techniques could be suggested depending the actual requirement of research advance. Indeed, recently, insight from (Mazzoleni *et al.*)describe the possible role of litter decomposition by-products accumulation in soil as possible species specific biomolecule acting particular role in Plant-soil feedback interaction. In light of this, should be plausible to think that through the strong enzymatic activity characterizing ECM fungus detrimental compounds could be heavily decomposed as potential sources of N and P available for plant growth.

Interestingly, such theory might be widely stressed though detailed techniques as such as Stable Isotope Probing. The aforementioned approach could be suggested as method to understand effective role of EM fungus in the acquisition of particular biomolecules from litter or root exudates describing peculiarities of nutritive cycle in ECM monodominant forests. However, despite the promising ability of SIP, literatures lacks of studies relating the effect of self-litter decomposition products and plant growth or lack information on the mechanisms in which this process proceeds in natural environments. This is maybe given to difficult EM fungi culture that are needed for production of mycorrhized seedling and in turn could be limiting in representation

of wide complexity of mycorryzal community present in rhizosphere. So far, EM associations still remain unclear and underestimated in relation to studies in community ecology regarding plant-soil interaction. The effective role of EM fungus in affecting organic matter cycle should be a helpful advance in the field of plant community ecology and could provide key information for restoration ecologists.

3.2. Interaction between ectomycorrhizal symbiosis, plant-soil feedback and soil microbiome.

3.2.1. Introduction.

Plant-soil feedback sciences are actually the study of how plant affect biotic and abiotic characteristics of soil, and how this, in remand, modifies the populations of the conditioning plants or those of other plants species. At the highest level, the feedback conditions triggered affect the whole plant community reaching landscapes scales (Van der Putten *et al.*, 2013). Among the wide variety of interactions described in this framework the plant-fungus symbiotic relations known as mycorrhizas assumes important role in configuring plant community structure (Van der Heijden *et al.*, 1998; Klironomos *et al.*, 2011).

Among the four different types of mycorrhizas the Ectomycorrhizas (ECM) forms one of the most extensive symbiotic relationship in terrestrial ecosystems (Brundrett, 1991). Formed mostly by Basidiomycetes that associates with woody plants, at biogeographic level ECM occurs often in temperate and boreal forests with few exceptions in well drained forests of the tropical belt (Brundrett, 2004; Corrales *et al.*, 2018). In ecosystems where ECM symbiosis is common the advantage for hosts plants is traduced in a positive plant-soil feedback (Bennett *et al.*, 2017). So far, the effects on the extant community structure is traduced in dominance of the plant species carrying the symbiosis (McGuire, 2007). In more detailed way, the advantage produced by ECM fungus to the plants is showed by the different pattern of renewal distribution with respect to non-ECM plant species. In non-ECM forests renewal in woody plants distributes following Janzen-Connell effect (Packer & Clay, 2000; Marchand *et al.*, 2019). Generally, Janzen-Connell effect is described as a decreased probability of survival of conspecific seedlings beneath the mother plants that is also reflected with an increased biomass of the more distant renewal (Janzen, 1970). Oppositely, in ECM forests, the presence of noncontemporary siblings, saplings and poles distributing in inverse way with respect to those described by Janzen and Connell in tropics suggest an important role of the symbiotic relationship in patterns of woodland community structuration (Henkel *et al.*, 2005).

Different hypotheses were formulated regarding the mechanisms behind the Janzen and Connell effect: Plant pathogen accumulation beneath mother plants (Packer & Clay, 2000), depletion of nutrients in species-specific niches (Bonanomi *et al.*, 2008; Smith-Ramesh & Reynolds, 2017) and allelopathic effect derived from negative products of self-produced organic matter decomposition (Bonanomi *et al.*, 2006b; Mazzoleni *et al.*, 2007; Mazzoleni *et al.*, 2015a). In the case of ECM monodominated forests a *hypothesis non fingo* suggest that the symbiosis act in the way in which one or all of these conventionally negative factors transmute in an enhancer of survival for nearest renewal. Accordingly, evident advantage is provided to seedlings by the symbiosis. ECM were documented to increase plant defences against pathogen through the structuration of a selective mycorrhizosphere and the increase of soil nutrient uptake for plants by means of the more efficient system of enzymatic secretion and adsorption provided from the ECM mycelium (ZengPu *et al.*, 1994; Tuomi *et al.*, 2001; Van der Heijden & Sanders, 2002). In reverse few studied focused in the ability of the ECM symbiosis to delete the allelopathic effect derived from self-deposed organic matter decomposition. In particular in its experiment Mazzoleni and coworkers in 2015 described a self-toxic effect from differently

decomposed conspecific leaf litter on germination and survival of the ECM-forming Oak *Quercus ilex* acorns and seedlings. The works described a new insight in plant soil interaction studies that however need to be verified in more real condition including the presence of an ECM symbiosis that characterize the species tested. Indeed, is evidenced from few works that ECM symbiosis triggers the ability, for both plant and fungus, to exploit leaf litter through the saprobic activity of the associated fungus (Perez-Moreno & Read, 2000; Read & Perez-Moreno, 2003), hypothetically shifting the effect described from Mazzoleni et al in a nutritive advantage for the plant carrying the symbiosis.

However, the lack of knowledge about the ability of the ECM symbiosis to decrease litter derived autotoxic effect transforming it in nutrients for plants lack in literature. moreover, most of works studied this effect as and interaction between plant and soil and not as symbiotic system and soil that is a strong bias considering that plants should be studied as and holobiont (Molter, 2019).

By this point of view, we consider the different response in morphometries and plant growth as important indicator of the relation between the symbiotic system and the variation of the condition of the surrounding environments operated by enrichment of self-leaf litter at different ages of decomposition.

Given this, in the present work we propose an experimental approach in order to observe:

i.- The variation in architecture of ECM plants to assess the role of self-organic matter enrichment in soil.

ii- The allocation of plant biomass in response to litter enrichment.

iii- The effect on plant architecture and biomass from litters at different decomposition stages.

iv- The ability of ECM symbiosis to shift from an autotoxic plant-soil negative feedback to a positive one.

3.2.2. Material and methods.

Collection plant material, fungus material and inoculation

All material was collected in the woodland of Parco Gussone (40° 48′ 40.3″ N, 14° 20′ 33.8″ E, elevation 75 m a.s.l.; about 1 km distant from the coast line) in the Bourbonic royal palace of Portici. The site is a naturalized artificial woodland with an age of approximatively 300 years from the plantation, with an average canopy height of 10 m and homogenous light radiations. The site is dominated by the helm oak *Quercus ilex* with few intermixing species at a coverage lesser than 10%. Other species present in the woodland with minor distribution are *Fraxinus excelsior* and *Laurus nobilis.*

Soil is shallow with andic properties, lays over pyroclastic deposits from eruptions of the Mount Vesuvius dated 1631. The climate is Mediterranean, with humid winter and dry summer, total yearly rainfall of 929 mm (290, 200, 89 and 348 mm in winter, spring, summer, and fall, respectively), and mean monthly temperatures ranging between 11 °C (January) and 26 °C (August) (Bonanomi *et al.*, 2018a).

The fungus material for inoculation was provided by ID Forest laboratories (http://idforest.es/, Spain) and consists in certified spores of *Pisolithus tinctorius*. *Q. ilex* acorns were collected according to whether acorns producing plants were also characterized by the presence of *Pisolithus spp* carpophores. Seeds was selected and those presenting damage, lesion, molds, or structural inhomogeneity was discarded. Selected seed was dried for few days and subsequently placed at a temperature of 4°C to vernalize, temperature treatment was carried out in order to avoid discontinuous germinations and given the xerophilic nature of the plant and

the Mediterranean biogeography a period of one day was considered enough. Prior to seed germination seed was sterilized 5 min in sodium hypochlorite at 1% concentration (v/v). Following sterilization, seed destined to inoculation was separated from those not destined to produce ECM symbiosis. For germination seeds in each group was placed in different plastic hermetic boxes with dimension of 60x40 for each sides and 40 cm of height. Substrate used for germination was sterilized and composed by peat and vermiculite in ratio 3:1. Germination of oaks was above ground and occur homogeneously 5 day after acorn placements. Seeds not germinated after a period of ten days was discarded. Inoculation phases was carried out at the Department of agricultural sciences of the Federico II University in Naples and consists in additions of 5 g of *P. tinctorius* spores to water used for irrigation. Inoculum was sprayed on the germinated seedlings and inoculation repeated for three times Each 4 days. After a period of 1-month seedlings was transplanted to experimental conditions.

Experiment 1

In order to isolate effect of decompositions of self-litter materials on ECM plants a full factorial design experiment has been carried out. We compare effect on total, aboveground and belowground biomass of ECM and non-ECM plants in presence or absence of self-litter. We also scrutinize the ability of ECM plants to decompose and get advantage from self-litter in presence and absence of a native microbial community (Figure 3.2.1). Hence, as soil conditioning factor we include supplement of conspecific fresh litter collected from mother plants and soil sterilization to exclude microbial community. Soils was collected beneath mother plants mixed and sieved in coarse mesh (5x5mm) to avoid detritus of high dimension. Soil destined to be used in absence of a decomposing community was sterilized in autoclave with standard procedures consisting in 3 cycle of sterilization at temperature of 121° for a time of 15 min. After sterilization inoculated and non-inoculated seedling of *Q. ilex* was transplanted from germination box to labelled pots. For a period of 3 days plants was covered by plastic bag to avoid hydric stresses from transplantation. Following self-litter conditioning was produced adding whole fresh leaves of mother plants in experimental soil surfaces for an amount of 2% of total soil volume. Litter sterilization was not carried out in order to avoid changes in biochemical composition of the organic matter subject to the studies. Experiment was carried out in pots with a diameter of 14 cm with a volumetric capacity of around 1 L of soil. Eight replicates of each combination of experimental factor was produced. Seedlings of *Q ilex* was growth for 13 months in greenhouse facilities of the department of Agricultural sciences and periodically watered at field capacity. After growth phases *Q. ilex* plants was sampled in order to obtain biomass data. Prior to this, plants were checked for presence or absence of ECM root tips. Plants inoculated but not presenting of ECM tips was discarded by statistical analysis.

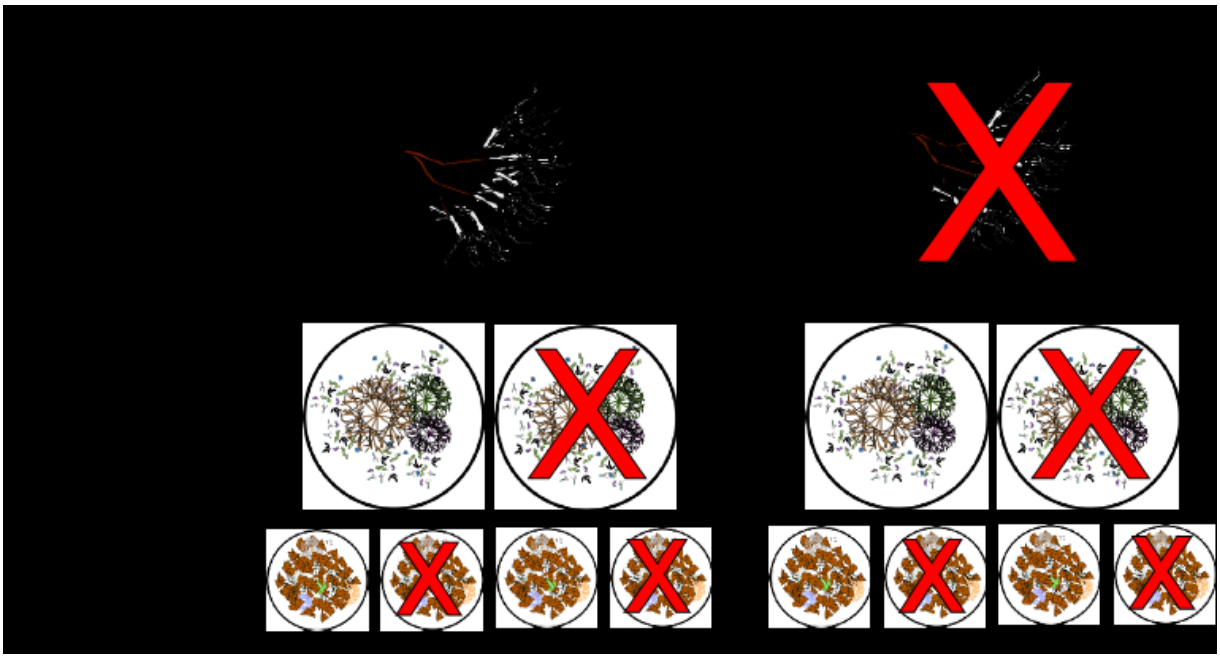

Figure 3.2.1 Experimental design of experiment 1. Experiment follow full factorial design including the combination of presence or absence of Ectomycorrhizal symbiosis in plants growing in sterilized or unsterilized soil with presence and absence of conspecific litter.

Experiment 2

After the first experiment we produced ECM and non-ECM seedlings as described for experiment 1. Seedlings was used to check the effect of self-litter supplements at different decomposition stages on roots growth pattern of *Q. ilex*. In order to visualize the direct effect on roots and provide continuous sampling during seedling development, experiment was conducted in one side transparent mesocosms. In detail, mesocosms consist in container of 100 cm depth x 15 cm width x 5 cm of thickness. One side of the container was in transparent plastics material, rather than, the other side consist in rigid, opaque polycarbonate layer. Narrower side consists in foam bands. The modules of the mesocosms was previously sterilized by washing with sodium hypochlorite 1% and rinsed with abundant water in order to avoid effect from residuals. Mesocosms was filled with soil originated and treated as the same way of the experiment 1. Experimental treatment was made adding 2% of litter on the total amount of soil (~ 2 kg). Treatment consist in addition of fresh litter from mother plant, addition of decomposed litter for a period of 120 days in laboratory condition and no addition of litter. For decomposed litter decomposition trial was made in 50x30x30 plastic trays, periodically watered, at a temperature of 20°C. To promote decomposition of leaf litter, a sample of soil was collected in correspondence of sampling point of leaf litter. Inoculum of soil was added using slurry methods according to litter decomposition in laboratory conditions (Bonanomi et al.2019). After litter supplement ECM and non-ECM seedlings of *Q. ilex* was transplanted. As treated for experiment 1, seedling was covered for a period of three day to avoid hydric stress of transplantation. Mesocosms was placed in oblique position of around 60° in order to promote roots gravitropic growth toward the transparent plastic layer and permits periodical data collection.

Experiment extended for the period needed to the roots to reach the basis of the mesocosm. After that period experiment was considered concluded in order to avoid that spatial limitation of mesocosms affect root growth and architecture. In that period, data regarding root spatial expansion, length and number was collected each 15 days.

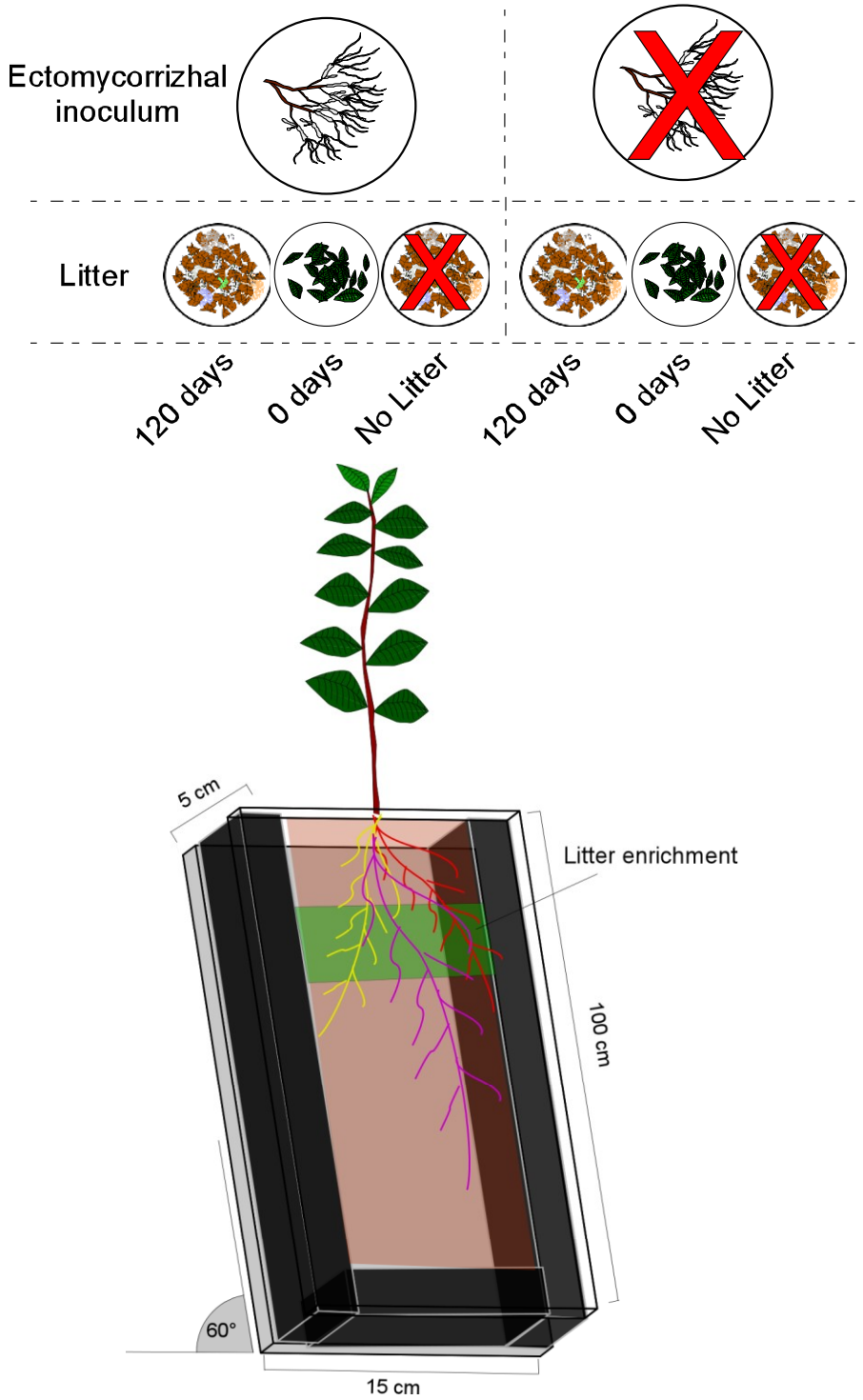

Figure 3.2.2 Upper panel) experimental design of experiment 2. Experiment follow full factorial design combining presence and absence of Ectomycorrhizal symbiosis in plants with three different litter treatment: No litter, fresh litter and decomposed litter. Lower panel) representation and size of the mesocosms used for the experiment. (representation is reversed respect experimental condition in order to increase understandability of the experimental setting)

Statistical analysis

For experiment 1 fresh biomass data was collected for both aboveground and belowground portions. Data was analysed by factorial analysis of variance and significance was assigned according to Duncan test at significance level of 0,05 *p-value*. In order to isolate effect of different treatments on ECM and non-ECM, total biomass data of *Q. ilex* seedlings was analysed through relative interaction index.

For experiment 2 data was compared for total root length, total length of fine roots and number of fine roots in seedlings growing in the presence or absence of an ECM symbiont associated to different decomposed litter by factorial analysis of variance and significance assessed by Duncan test at p-values below 0.05. Moreover, the increase of length and number of fine roots was analysed to test the effect of the presence of symbiont, presence and absence and period of decomposition of self-litter added and the date of sampling. Other parameter explaining root strategical proliferation and the trajectory assumed across the date so sampling was analysed by principal component analysis (PCA). All statistical analysis and plotting were performed using Statistica 10 software.

3.2.3. Results.

Experiment 1

Total Biomass, root biomass, shoot biomass and Root-shoot ratio was measured in *Q. ilex* seedlings inoculated and non-inoculated with *P. tinctorius* spores in combination with self-litter enrichment and sterilization. Bar plots showed a generalized absence of significant variation when considering independently the mycorrhizal state, the presence or absence of a soil microbiota and the presence of self- litter (Figure 3.2.3).

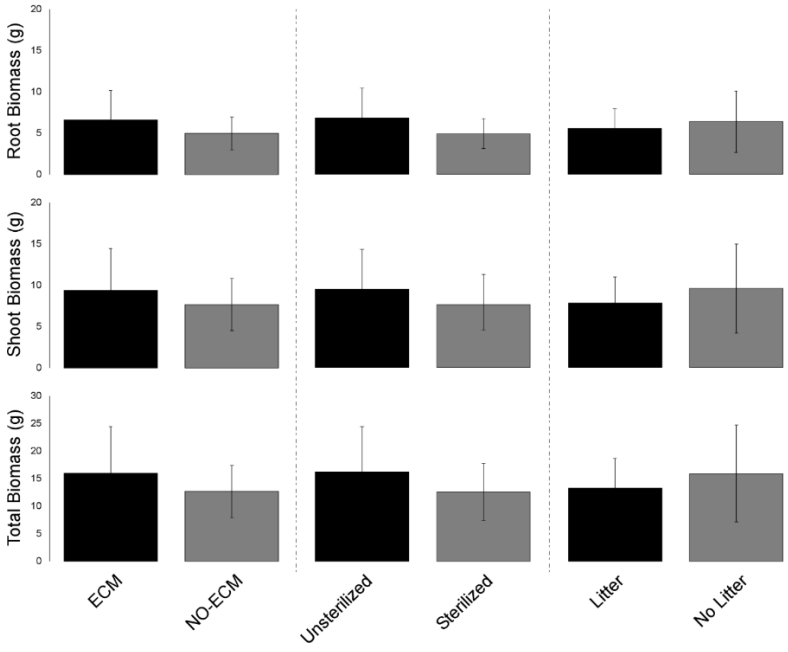

Figure 3.2.3 bar plot of root biomass (upper panel), Shoot biomass (middle panel) and Total biomass (lower panel) considering the singular effect of the treatments in the experiment.

Differently, when considering the interactive effect of the treatments, significant variations are observed for both, root biomass, shoot biomass and total biomass (Table 3.2.1). In detail, increased level of growth was observed for ECM plants in unsterilized soils. Among the ECM plants, those treated with a supplement of self-litter showed the higher level of growth compared to all the other treatment. While, no significative changes are observed in Root-shoot ratio values (Figure 3.2.4).

Table 3.2.1 GLM (Generalized linear model) testing significant variations for root, shoot, total biomass and root/ shoot parameters for *Quercus ilex* seedlings growing in presence of Ectomycorrhizal symbiosis of *P. tinctorius*, litter and sterilized soil. Significant values in bold with *p-value* below 0,05

Treatments	Root biomass		Shoot Biomass		Tot Biomass		Shoot/Root Biomass	
	F	*P-value*	*F*	*P-value*	*F*	*P-value*	*F*	*P-value*
Intercept	**306,5646**	**0,000000**	**275,5821**	**0,000000**	**321,0713**	**0,000000**	**709,3360**	**0,000000**
ECM inoculum (ECM)	**7,7666**	**0,007709**	3,1206	0,083943	**5,2373**	**0,026750**	1,4969	0,227373
Sterilization (ST)	**9,3355**	**0,003734**	3,4724	0,068791	**6,0607**	**0,017630**	0,7087	0,404244
Litter (LT)	1,8658	0,178597	2,9363	0,093342	2,7795	0,102276	0,0119	0,913547
ECM x ST	**16,1402**	**0,000216**	**13,1276**	**0,000724**	**15,9348**	**0,000234**	0,0015	0,969246
ECM x LT	0,1631	0,688187	0,0502	0,823699	0,0968	0,757136	0,0313	0,860287
ST x LT	1,1878	0,281445	0,9591	0,332537	1,1677	0,285501	0,1795	0,673811
ECM x ST x LT	3,3740	0,072700	2,3372	0,133167	3,0376	0,088036	0,0850	0,771999

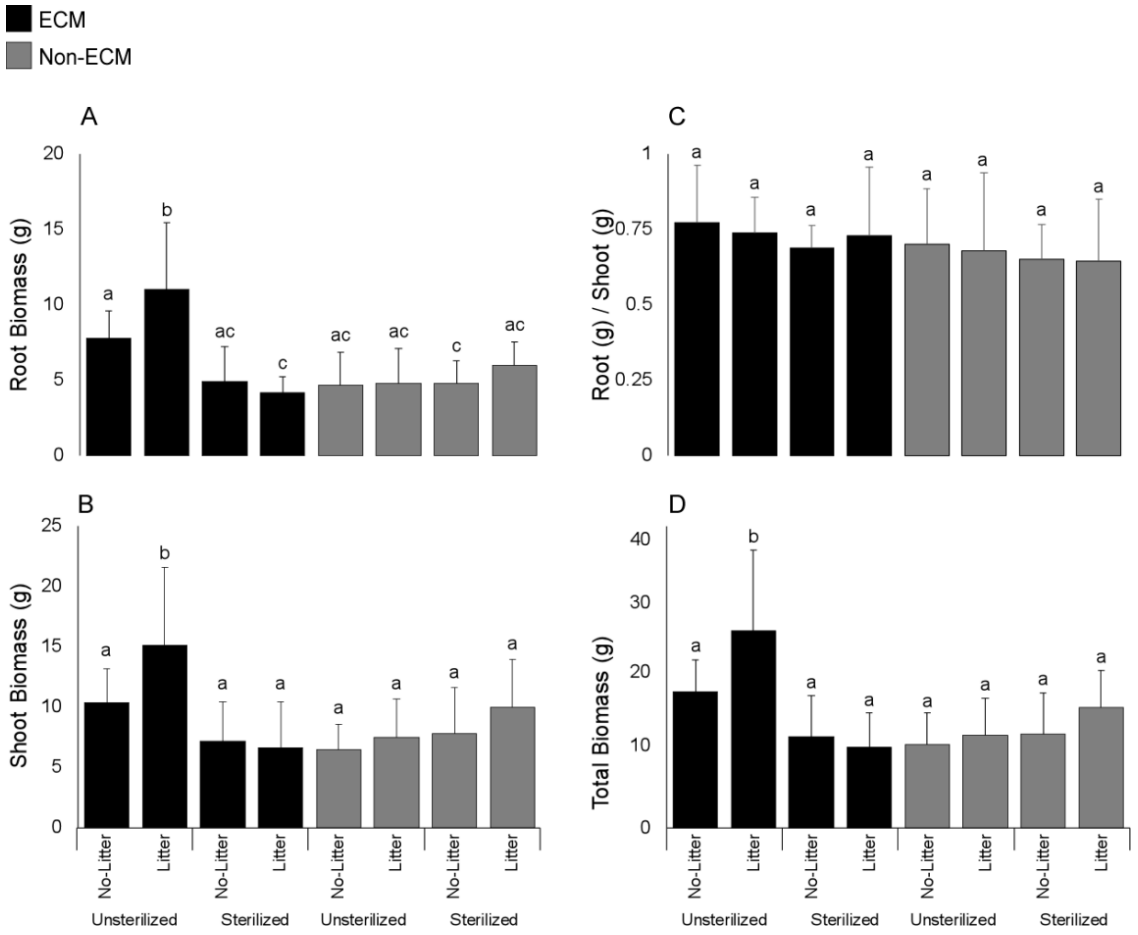

Figure 3.2.4 bar plot showing root (a), shoot (b), total biomass (d) and root/ shoot (c) values for *Q. ilex seedlings* growing in all the combination of the three experimental conditions (presence of Ectomycorrhizal symbiosis, presence of litter and soil sterilization). Lecter indicate significant difference between each experimental unit. Significance was assigned according to Duncan *post-hoc* test for *p-values* below 0,05.

The analysis of Relative interaction index (RII) reveals that the comparison among the different treatment operated on *Q. ilex* seedling, the ECM inoculation and the removal of litter promoting growth of seedlings. Oppositely negative values are observed for the process of sterilization becoming a growth suppressor (Figure 3.2.5). When considering litter removal and sterilization as processes affecting the growth of ECM and non-ECM plants, RII reveals that for Non-ECM plants litter removal and sterilization act as growth promoting practice. Differently, in ECM plants, the effect of sterilization strongly affected in negative way the development of seedlings (Figure 3.2.6).

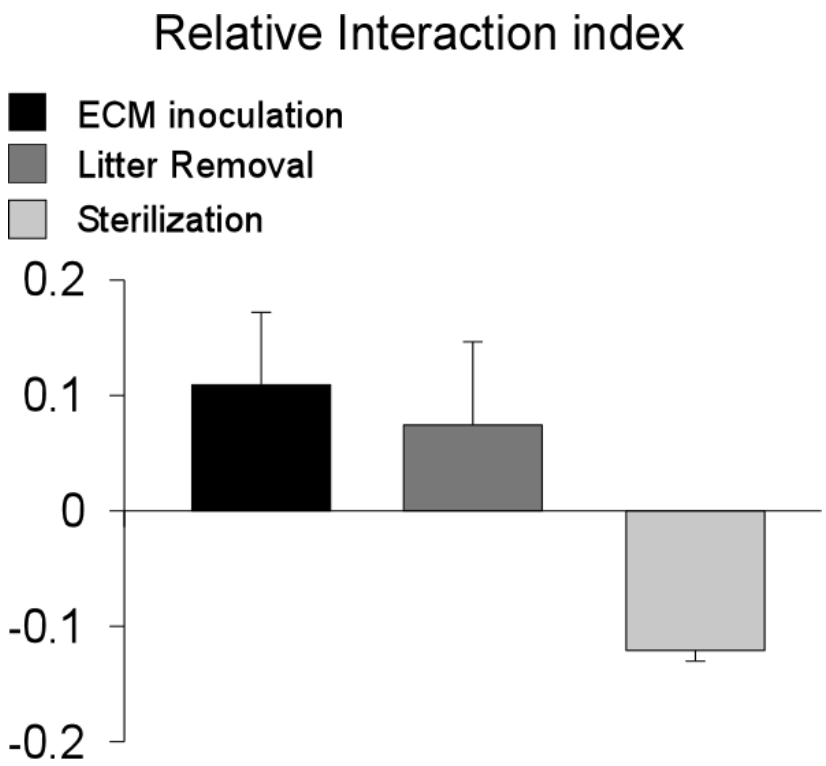

Figure 3.2.5 RII (relative interaction index) explaining the effect of Ectomycorrhizal symbiotic inoculation of *P. tinctorius*, Litter removal and soil sterilization on growth of *Q. ilex* seedlings.

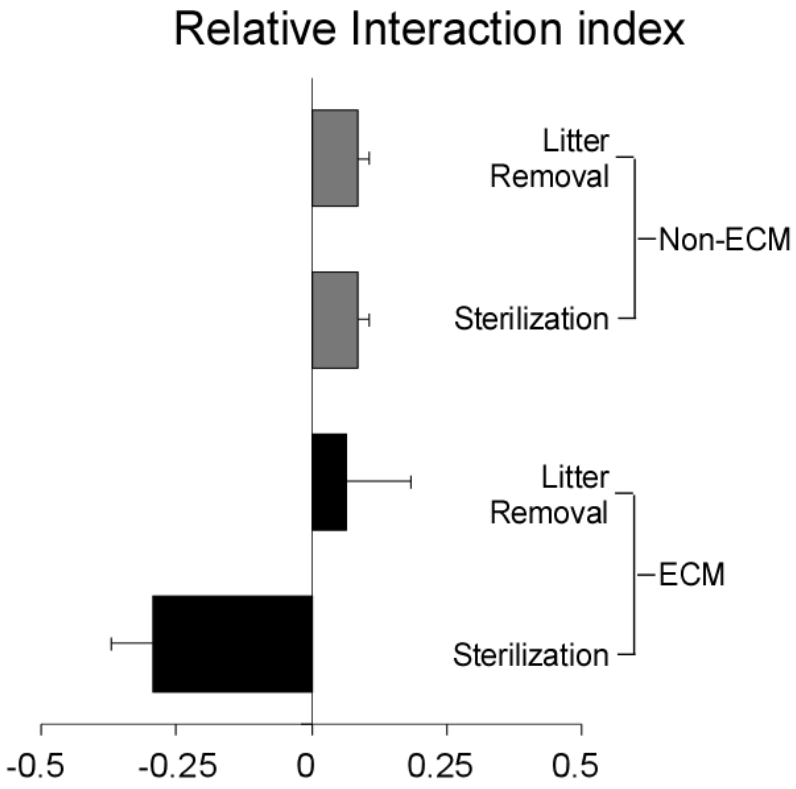

Figure 3.2.6 RII (relative interaction index) showing the effect of soil sterilization and litter removal on *Q. ilex* seedlings in presence and absence of ectomycorrhizal symbiosis with *P. tinctorius*

Figure 3.2.7 detail of the ectomycorrhizal symbiosis in *Q. ilex* seedlings, in the upper photo is showed a portion of the root system of *Q. ilex* with presence of ectomycorrhizal root tips in unsterilized soil. In the lower photo is showed the root system of ectomycorrhizal *Q. ilex* seedlings in sterilized soil.

Experiment 2

Significant changes in roots morphometry and architecture was observed comparing ECM and Non-ECM seedlings of *Q. ilex.* At the end of the experiment variable level of growth has been observed between ECM plants and Non-ECM plants and according to litter supplement typology.

Among ECM seedlings, higher level of root length was observed for plants growing in undecomposed and decomposed litter (120 days) while those growing in absence of root supplement has depressed growth compared to the other. Oppositely, root growth observed in Non-ECM plants was inhibited by the presence of fresh and decomposed litter and enhanced in absence of this. Noticeably, when growing without litter, Non-ECM plants has average values of comparable to those of litter enriched ECM plants. Generally, the trend described is repeated for measured variables as such as total root length, total root length of fine roots and number of fine roots (Figure 3.2.9).

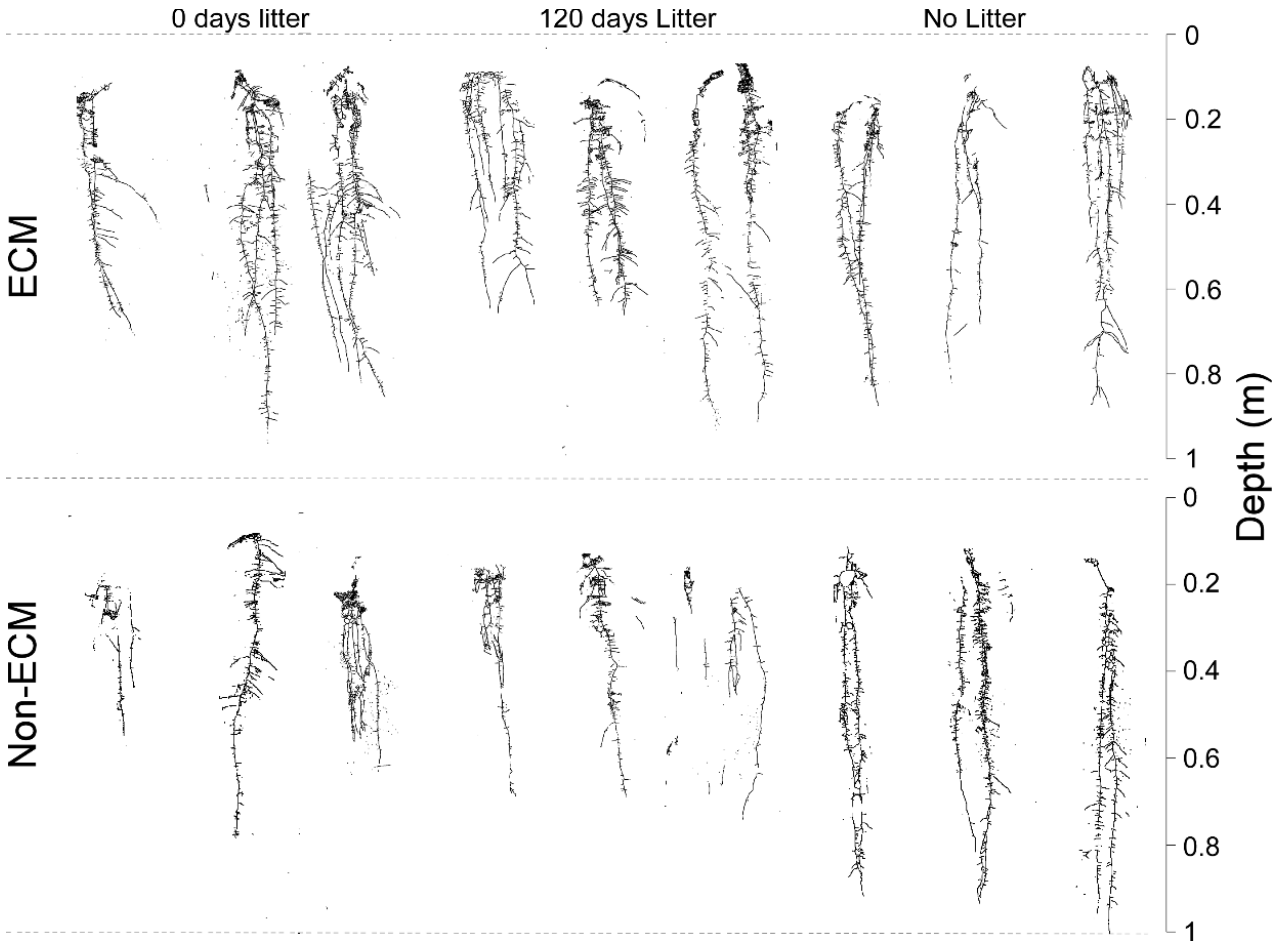

Figure 3.2.8 Root systems of *Q. ilex* seedlings growing in presence (up) and absence (down) of ectomycorrhizal symbiosis of *P. tinctorius* with three different type of litter regimes: fresh litter (0 day decomposed), aged litter (120 day decomposed) and without litter.

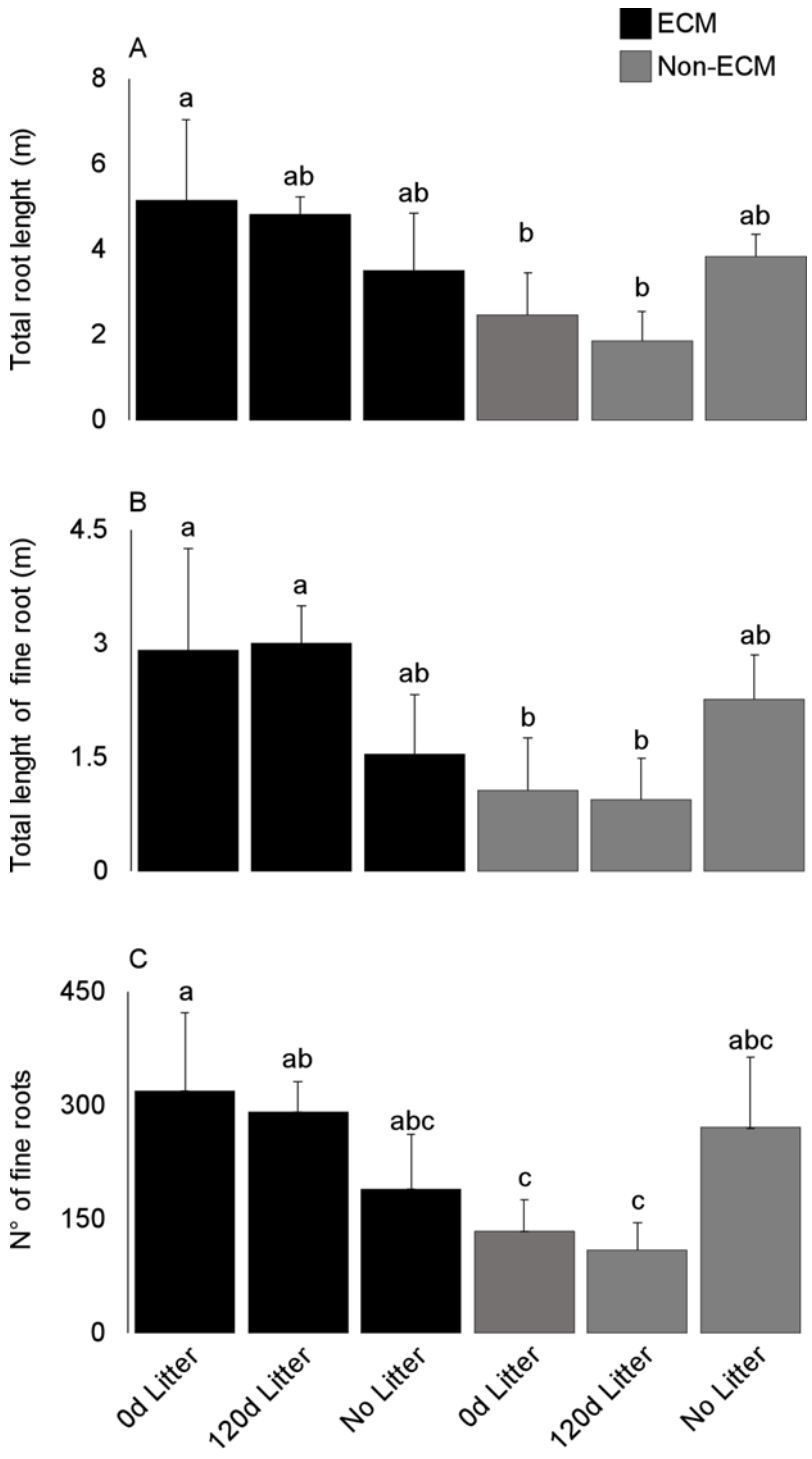

Figure 3.2.9 Bar plot showing a) total root length, b) total length of fine roots and c) N° of fine root values in *Q. ilex* seedling growing in presence of *P. tinctorius* ectomycorrhizal partner with fresh litter, aged litter and with no litter. Letters indicates significant variation according to Duncan post-hoc test. Significance p-values below 0.05

The periodical measurement of root development showed that ECM plants are faster in producing roots being characterized by higher radical elongation when growing in litter enriched soil. Oppositely, Lower level of elongation is assessed in ECM plants growing without litter. Among Non-ECM plants, seedlings growing in non-enriched soil developed root metrics with similar values of those of ECM plants for the whole period. Cumulative growth trends showed that No-ECM seedlings developed best without litter starting from

the first stages of growth until the end of the experiment. Inversely, characteristic trends are observed in Non-ECM plants growing in decomposed litter, showing root development in the initial part of the experiment higher with respect to the other sampling points, where growth are stunned or reduced compared to the beginning.

The negative trends of root parameters in time is observed uniquely in plants growing in presence of decomposed litter and indifferently by the presence of ECM symbiont. In detail, the decline of root elongation and proliferation is observed at the second date of sampling (1 month) in ECM-plants, rather than Non-ECM plants has a decreased ability to synthesize new roots after the first date of measurement (15 days) (Figure 3.2.10).

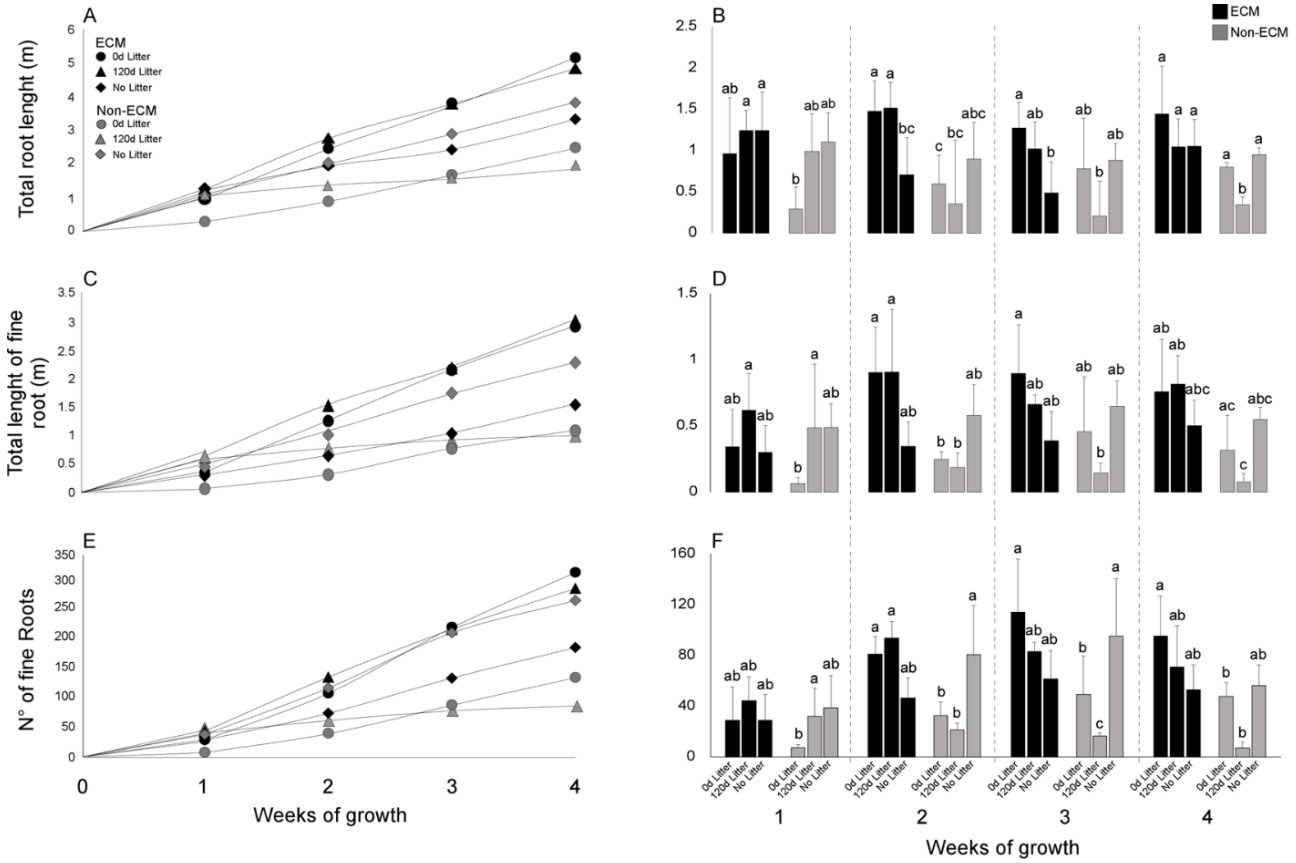

Figure 3.2.10 cumulative growth of *Q. ilex* seedlings for total root length (a), Total length of fine roots (c) and N° of fine roots (e). gain of total root length (b), Total length of fine roots (d) and N° of fine roots (f) measured each 15 days in ectomycorrhizal and non-ectomycorrhizal seedlings f *Q. ilex* with different litter regimes. Letters indicate significant variations assigned through Duncan post hoc test (p-value < 0,05).

Table 3.2.3 GLM (Generalized linear model) testing significant variations in total length of fine roots, Number of fine roots and total root length of *Q. ilex* seedlings during a period of two months in presence of ectomycorrhizal symbiosis of *P. tinctorius* and conspecific litter at different stages of decomposition.

treatments	Total length of fine roots		N° of fine roots		Total root length	
	F	***p***	***F***	***p***	***F***	***p***
Intercept	**252.8456**	***0.000000***	**382.8198**	***0.000000***	**432.5799**	***0.000000***
ECM	**19.0983**	***0.000066***	**22.7757**	***0.000017***	**25.5213**	***0.000007***
Litter	0.0475	*0.953665*	1.7781	*0.179905*	0.5802	*0.563646*
week of growth	1.3507	*0.269108*	**9.4895**	***0.000050***	1.0156	*0.394054*
ECM*Litter	**13.5504**	***0.000022***	**18.0763**	***0.000001***	**9.1856**	***0.000419***
ECM*week of growth	1.4087	*0.251707*	1.2899	*0.288606*	0.6407	*0.592594*
Litter*week of growth	1.6025	*0.167024*	1.9464	*0.092323*	**2.7618**	***0.021824***
ECM*Litter*week of growth	0.5342	*0.779553*	1.2259	*0.309768*	0.9316	*0.481133*

Moreover, eight parameters were measured to obtain information on root architecture and strategy of proliferation in soil. Then data was reduced by means of Principal component analysis (PCA) explaining around 90 % of the total variance in the data (PCI: 68,44% and PCII 21,65%). Despite higher level of variance is explained on the first principal component, because of the effect of root growth, differentiation in the disposition of variables in the second principal component appear to be the more explicative of the patterns of root proliferation and strategy. More precisely, the multivariate analysis showed that ECM plants are generally more correlated to the development of a wider root apparatus and higher ellipse area. Same strategy is assumed by litter enriched non-ECM plants that developed wider root apparatus but with lower correlation because of the scant growth. Complete inverse strategy is developed in Non-ECM plants growing with higher depth-width ratio, higher density of fine roots and higher depth (Figure 3.2.11).

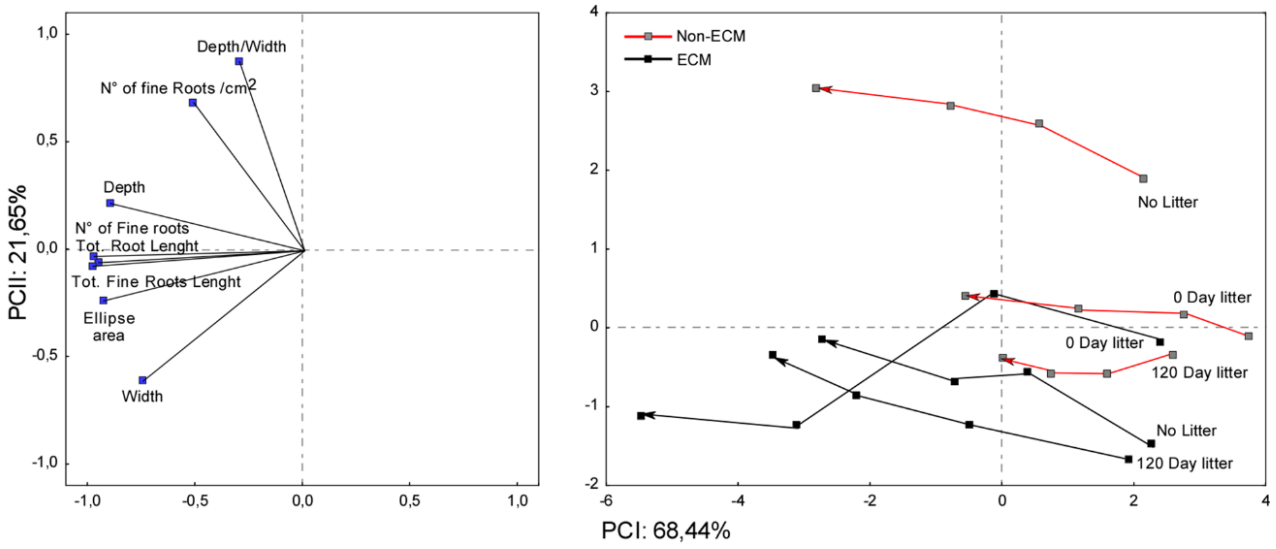

Figure 3.2.11 Principal component analysis (PCA) explaining variation in root architectures associated with ectomycorrhizal and non-ectomycorrhizal *Q. ilex* seedling growing with different litter regimes. In right side score plot of the variable measured to explain root system architectural changes. Left-side trajectories of associated loadings in each sampling date.

3.2.4. Discussion.

In the present experiments we demonstrate importance of the relation of *Q. ilex* seedlings with one of its elective fungal symbionts. Moreover, we observed that the effect of the symbiotic relationship formed became advantageous for plant establishment only when in the presence of a stable soil microbiome. We also observed that the plant-fungus relationship became more positive in presence of conspecific-litter. Taken together, these evidences lead us to hypothesize that the positive outcome for establishment and growth of symbiotic plant is an interplay between the natural soil microbiome and the presence of self-litter that is probably connected in an organic matter decomposition process. The results are in accordance with studies demonstrating that late stage decomposed litter suppress *Q. ilex* growth at conspecific level when elective fungal symbiont are not present (Mazzoleni *et al.*, 2015a). Inversely, is demonstrated that with ECM symbiosis the detrimental effect of litter is reversed in accordance with those work underline the positive effect of ECM symbiosis on plant establishment in conspecific conditioned soils (Bennett *et al.*, 2017; Teste *et al.*, 2017).

In our experiment we also observed that ECM seedlings growing in sterilized and unsterilized soil developed a different morphology of plant-fungus relationship. Whether ECM plants in unsterilized soil showed visual evidence of the formation of ECM structure. Surprisingly, in sterilized soil the symbiotic interaction assumes different morphological state consisting in an expanded mycelial net surroundings also suberized portion of roots (Figure 3.2.7). The presence of this particular morphological condition uniquely appeared in the ECM plants that undergoes the treatment and reminds to characteristic formation of dense saprobic mycelium decomposing organic matter. The observation of an unusual development of mycelium near *Q. ilex* roots is incidentally associated with a depressed growth of oak seedlings. Putting together the information from the experiment, we suspect that the presence of a microbial community regulates the symbiotic relation between plant and fungus avoiding the possibility that the fungal partner of the symbiosis should face a trade-off

between symbiotrophism and saprotrophism-pathotrophism again its host (Garbaye *et al.*, 1992; Garbaye, 1994; Frey-Klett *et al.*, 2007).

Few assumptions could be generated in the first experiment with the attempt to explain mechanisms by which soil microbiota is crucial for the positivity of the ECM symbiosis. However, the increase in growth promoted by the treatment with litter lead us to hypothesize that microbial community act as key to unlock nutrient immobilized in leaf litter (Garbaye, 1994; Dix, 2012). Subsequently, the action operated by the soil microbiome lead the possibility to the ectomycorrhizal basidiomycetes to release its enzymatic arsenal, that is developed for the decomposition of detrimental compounds (Baldrian, 2008; Rytioja *et al.*, 2014). Is acknowledged that during microbial succession almost the totality of basidiomycetes occurs at the end of the process of decomposition (Frankland, 1998; Purahong *et al.*, 2016; Bonanomi *et al.*, 2019). This since, whether in some way basidiomycetes has high diversity of enzymatic products and secondary metabolites, on the other hand, they lack in the ability to decompose and adsorb the labile compounds in fresh deposed organic matter (Deacon, 2013). So far, could be assumed that the dependence by the microbial community is because it acts as starter for basidiomycetes action and in turn advantage for symbiotic plants (Berg & Ekbohm; Hiscox *et al.*, 2018).

In the first experiment is observed that ectomycorrhizal symbiosis function well in unsterilized conditions. The suggestion raised in that trial provided useful information for the understanding of ECM Symbiosis functioning. The full comprehension of the process still requires deeper investigation but underline the importance of a proper microbiome in soil for the development of important processes as such as ECM symbiosis in woodland soil. In a different framework, the second experiment provide us a detailed view on the effect of ECM plants growing in presence of litter (differently decomposed) and the effect on plant root proliferation and strategic expansion. We do not apply root sterilization because already stated the essential need of a structured soil microbiome supporting plant growth. Subsequently, the results of our experiment demonstrated that ECM relationship became more positive in presence of self-litter that presumably is used as nutrient resource and, inversely, when ECM symbiosis is not present, self-litter has a detrimental effect on root proliferation with a more probable phytotoxic effect (DeBell, 1971; Dabral *et al.*, 2018). This is also confirmed by the values reported in plant growing in absence of litter enrichment that in ECM condition appear to be depressed, meanwhile without symbiotic inoculum is promoted to positive levels. To date, when comparing fresh and decomposed self-litter, phytotoxicity became higher at 120 day of decomposition. This effect was similarly demonstrated in work on germination of *Q. ilex* acorns with decomposed self-litter underlining the presence of a species-specific self-detrimental effect from the release of autotoxic self-DNA (Mazzoleni *et al.*, 2015a; Mazzoleni *et al.*, 2015c). These evidences allow us to think at a similar effect, that is however present but less effective in ECM seedlings when compared to ECM seedling growing in correspondence of fresh litter.

More interestingly, root development in the four date of sampling showed that almost the totality of seedlings has a decreasing ability to allocate biomass in root. Only exception is made for Non-ECM seedlings growing without litter. This is demonstrated by point of inflection of the trend. Indeed, in ECM plants the inflection points commonly take place at 30 day of growth rather than Non-ECM plant do not showed inflection

but a continuous negative development from the first date of sampling until the end of the experiment. This suggest that negative condition for root expansion appeared later in ECM plants as it is possible that the phytotoxic compounds from litter, presents in the first layer of soil, are early degraded by the fungal mycorrhizal partner. We consider this effect on the first layer of soil because of the low effectiveness of the ECM hyphal activity in the lower layer of soil (Dickie *et al.*, 2002; Rosling *et al.*, 2003). Inversely in Non-ECM plants phytotoxicity became early present as probably lacking enzymes produced by the Basidiomycetes acting as chemical degrading agent in the upper layers of soil.

Regarding how ECM symbiosis act in positive way for *Q. ilex* seedlings and how the energy obtained in the first layer of soil is transferred to root structures, our analysis of the root system evidenced that ECM plants has a wider spread of the adsorbing system with respect to Non-ECM seedling. This strategic adaptation is particularly evident when comparing Non-ECM plants growing in absence of self-litter that evidentially prefer to growth in deeper soil layer with no lateral ramification or genesis of lateral leading roots. Concomitantly is observed and increase of fine root density in the main leading roots, overly magnifying in a bifidum herring bone structure. This structural development could be interpreted as structural development triggered by an environmental context where perturbation or agent inducing root structural modification are minimized. Additionally, the downward exploring behaviour of these root structures could be given to the characteristic evolutionary fitness of Mediterranean *Q. ilex* that preferentially explore deeper layer of well hydrated soil (David *et al.*, 2007). So far, the gravitropic and water-dependent polarity of root appear to be highly preserved with respect to other plants that, although has certainly this kind of effect, preferentially developed for the expansion of the root system in a wider area. The effect could be interpreted in different way: In the first instance is probable that the structure of the root system undergoes earlier modification by the increase in ramification promoted from ECM roots and because the ramification on ECM plants lead to the production of higher number of leading roots. As consequence by a general self-repulsion and intraindividual competition root system assume a wider arrangement. This could also be supported by the higher level of resourced scavenged by the ECM symbiosis that can support a more extended and articulated root system. On the other hand, although in minor extent, Non-ECM plants preferentially developed a root system with a preferential wider expansion but only in presence of litter. This, bring us to a consideration that the increased expansion could be generated by the loss of apical dominance in the early stage of root development triggering ramification and dominance of lateral roots. To date, the effect of loss of apical dominance could be assigned to a direct phytotoxicity effect on root apex becoming sensible to phytotoxic compound released from litter. The explanation of the phenomenology could rely on both the hypotheses but do not explain the whole strategic development of the root system. Indeed, whether ECM plants could produce wider root system because of the increased tenor of resource acquisition, the biases on the assumption coming directly by the ability of the Non-ECM plant to produce a preferential wider root system. On the other hand, the self-litter damaging hypothesis could be partially accepted because of the presence of a wider root system in ECM plant growing in absence of litter. Conceivably, the two mechanisms hypothesized, could be interpreted as a mixed effect of both the processes of increased ramification in ECM plants and loss of apical dominance by phytotoxicity.

3.2.5. Conclusions.

Putting the results in a plant ecology framework, we are aware that survival of renewal in temperate forest is more dependent by the availability of light for the production of photosynthetic products with respect to the formation of a favourable microbial relationship as symbiosis (Brown *et al.*, 2019). This concept appears to be true whether considering that successful renewal in woodland ecosystems completely depend by formation of canopy gaps or only take place in woodland borders (for example Woodland-prairie ecotones)(Brown *et al.*, 2019). However, when light became a limited resource the distribution of renewal became crucially dependent by what its soil can provide and the direction of these interaction. Within the telluric framework, our results showed that positive ECM relationship formation and development of particular pathway in plant community ecology rely on more than one factor. The interplay between the microbes and litter affecting seedling growth suggested that the presence of a decomposing community in soil is fundamental for formation of monodominated ECM forest, this became possible because an increased ability to plant to scavenge resources both from the fungal partner but also by the higher surfaces of absorption and wider rhizosphere developed in those plants, that in other words means increased competitive ability with respect to Non-ECM species.

Here, our speculations are partially supported by the experimental condition proposed mostly because are described only the more evident portion of the relation between ECM symbiosis and litter decomposition. However, the experiment was carried out in soil, producing important information in a semi natural context in a more interactive background where the laboratory simplification can overemphasize or invert some results. More detailed information could be provided by future investigation aimed to detangle the complexity of soil organic and inorganic chemistry and the peculiarity of the microbial community present in there.

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4. Microbial succession in litter decomposition affecting plant community structure in grasslands

Overview

Fairy rings are generated by the expansion of fungal fronts of basidiomycetes outward from the point of origination. These biological phenomena are common in plant ecosystems and induce several changes in soil physical and chemical characteristics and changes in biotic communities of soil as well as plant community organization. The monodominance in basidiomycetes in soil is triggered by the release of organic matter in soil from the extant plant community in the late stages of decomposition. the particular effect of fairy rings has been extensively studied in the past and the results of these studies were limited by the technological improvement of the period or the lack of completeness in the experimental design or absence of true replications. Here we studied the bibliography concerning fairy rings studied from the last review in 1917 to nowadays and we focalize on the main lacks in literatures explaining this entangled biological phenomenon. We define surveys in order to study the extent to which fungal front of basidiomycetes alter the biotic community in soil and how it can be defined in a framework of driver of species richness in grassland. We attempted to relate the changes in the bacterial community with those of the physicochemical characteristics of soil and finally we focused the attention on the direct interaction between the fairy ring forming fungus and the plants by microbiological assay. We observed that fairy ring fungus of *Agaricus arvensis* acts as ecosystem engineer in grassland ecosystem studied by inducing perturbation in soil and promoting the restructuration of plant, fungal and bacterial communities. Particularly the fungal fronts of basidiomycetes formerly act in a detrimental way and then favour specific microbiota as in the case of *Trichoderma, Burkholderia* and Arbuscular mycorrhizal fungi. The detrimental effect of the fairy ring fungus is confirmed at multispecific level as it is present also in fungal fronts formed by *Calocybe gambosa*. Particularly, in there is observed a marked effect on the fungal community compared to the bacterial community that is more resilient because of specific association of bacteria of the phylum of Bacterioidetes getting advantage by the presence of the basidiomycetes. Moreover, is observed that the changes in soil microbiota triggered by the fungus are mainly triggered by changes in Organic matter, NH_4, NO_3, Mg, Fe, P in induction of Hydrophobicity. More in detail, we observed that the detrimental effect of the fungus on the vegetation is not formed by the release of volatile cyanuric compounds. More interestingly is observed that release of VOCs from the fungus has a stimulating effect on plant germination. These results underline the importance of study fairy rings as they can shed light on the complexity of the interaction between and within plant and soil microbiota in a natural experimental system. Moreover, as same levels of hail, frost, fire, grazing and autotoxicity, the expansion of fungal fronts acting as ecosystem engineer could be considered as important factor structuring plant community in grassland ecosystems.

4.1. Reviewing A century of study on fungal fairy rings

4.1.1. Introduction

Fungi are essential players in structuring terrestrial ecosystems functioning. Their activities includes direct role in biogeochemical cycle and an significant function in structuring plant community assemblages acting as pathogens, saprotrophs or symbionts (Dix, 2012; Paul, 2014; Dighton, 2016). As ubiquitous kingdom, fungi assume different forms and specific interactions depending by species or the environments in which develop. Because of their cryptic nature telluric fungi are hardly appreciable as important actors in terrestrial ecosystems. Indeed, fungal ecology and biology represent a tough topic for terrestrial ecologists attempting to elucidate the way in which and the extent to which fungus interact with the surrounding ecosystem (Bridge & Spooner, 2001). Clear cases in which a fungus mediate ecosystem functioning and characteristics are fungal Fairy Rings (FRs). FRs are described in grassland ecosystems as circular patterns of greener vegetation because of their changing effect on plant growth and color. Even in woodlands are recognized by the circular disposition of fungus carpophores. These kinds of phenomenon are produced by belowground expansion and activity of vegetative mycelium, and in some case, the astonishing regularity of carpophores disposition brought to the origination of several folk beliefs in old world, evocating magic or religion (Shantz & Piemeisel, 1917) and literature within). The actual name of "Fairy Rings" in which such ecological patterns is commonly recognized is the heritage of those folk beliefs.

Early attempts to describe the phenomenon were carried out by several ecologists, particularly in grasslands, where FR detection does not rigorously involve the presence of carpophores (Wollaston, 1807; Evershed, 1884). The reasons behind circular patterns of atypical vegetation in grassland were formerly hypothesized being due to quite a few of diverse explanations: i) activities of telluric mammalians, ii) work of ants, iii) release of feces and urines by herbivores. Finally, the presences of fungal mycelium growing in correspondence of changing vegetation were identified as causative agent. Comprehensively, such works were gathered in a review proposed by Shantz and Piezemel in 1917 in which the authors provide a comprehensive assessment of shapes, mechanisms and definitions that are still adopted in actual literature concerning FRs. Three main conclusion arise from that pivotal work on FRs: i) FRs are commonly formed by Basidiomycetes fungi, affirmation supported by the list of fungal species detected to forms FR in the preexistent literature; ii) FRs could assumes different shapes as continuous or fragmented circles and arcs; iii) The passages of growing mycelia generate physical and chemical changes in soil affecting plant community composition and productivity.

After a century from the publication of the first and last review of Shantz and Piezemel, scientific interest posed in FRs appeared to be multidirectional. Whether on one side, the scientific effort is carried out to detangle ecological significance and functionality of FRs in grassland ecosystems (Edwards, 1984; Bonanomi *et al.*, 2012; Espeland *et al.*, 2013; Hearst *et al.*, 2013), on the other side the role as turf grass pathogen of FR species has been extensively scrutinized (Halisky & Peterson, 1970; Terashima *et al.*, 2004; Fidanza *et al.*, 2007; Miller *et al.*, 2011). More recently, the advantage from the possibility to clearly identify the area of fungal dominance was used in order to isolate beneficial bacterial taxa that may support the intrigued

and difficult process of the formation of the ectomycorrhizas for commercial and forestry purpose (Kim *et al.*, 2014; Oh *et al.*, 2016; Oh & Lim, 2018a). Finally the potential role of "fairy chemicals" as plant hormones involved in increase of crop productivity become nowadays a promising area of scientific research and application (Choi *et al.*, 2017; Kawagishi, 2018). This review addresses the importance of studying FR formation, development, and effect on the surrounding environment as we consider it as a biological phenomenon with significant impact in ecosystem processes. Here we summarize the advancement in the knowledge of FRs starting from the review of Shantz and Piezemel until the present day.

4.1.2. FFRs classifications

Several authors proposed different classifications for FRs depending whether: i) a FR is generated by a symbiotic or a saprobic fungus, ii) according to the effect of the FR on grassland vegetation and iii) the configuration that the FR fungus assumes in soil horizon. These kinds of classifications, does not exclude each other because applied in different topics and because emphasize important characteristics of the phenomenon in different backgrounds.

The most detailed and widely applied classification was proposed by Shantz and Piezemel in 1917 for grassland FRs. The discerning factor in the classification was dependent by the qualitative changes produced by the FRs in vegetation cover. The authors grouped FRs in three typologies: The Type I FRs that are recognized by two zones in which fungus changes vegetation. A first area, more external, where the FR fungus produce a narrow belt of barren soil or inhibited vegetation followed by a second internal belt, with enhanced plant growth highlighted by a dark greenish color when compared with the other vegetation in grassland. The Second FR typology is that known as Type II FRs which is highlighted by the presence of a belt of darker vegetation with enhanced growth but without zones of necrotic vegetation. Finally, the Type III FRs do not show apparent changes in vegetation and are revealed seasonally by the emergence of fungus carpophores (Figure 4.1.1).

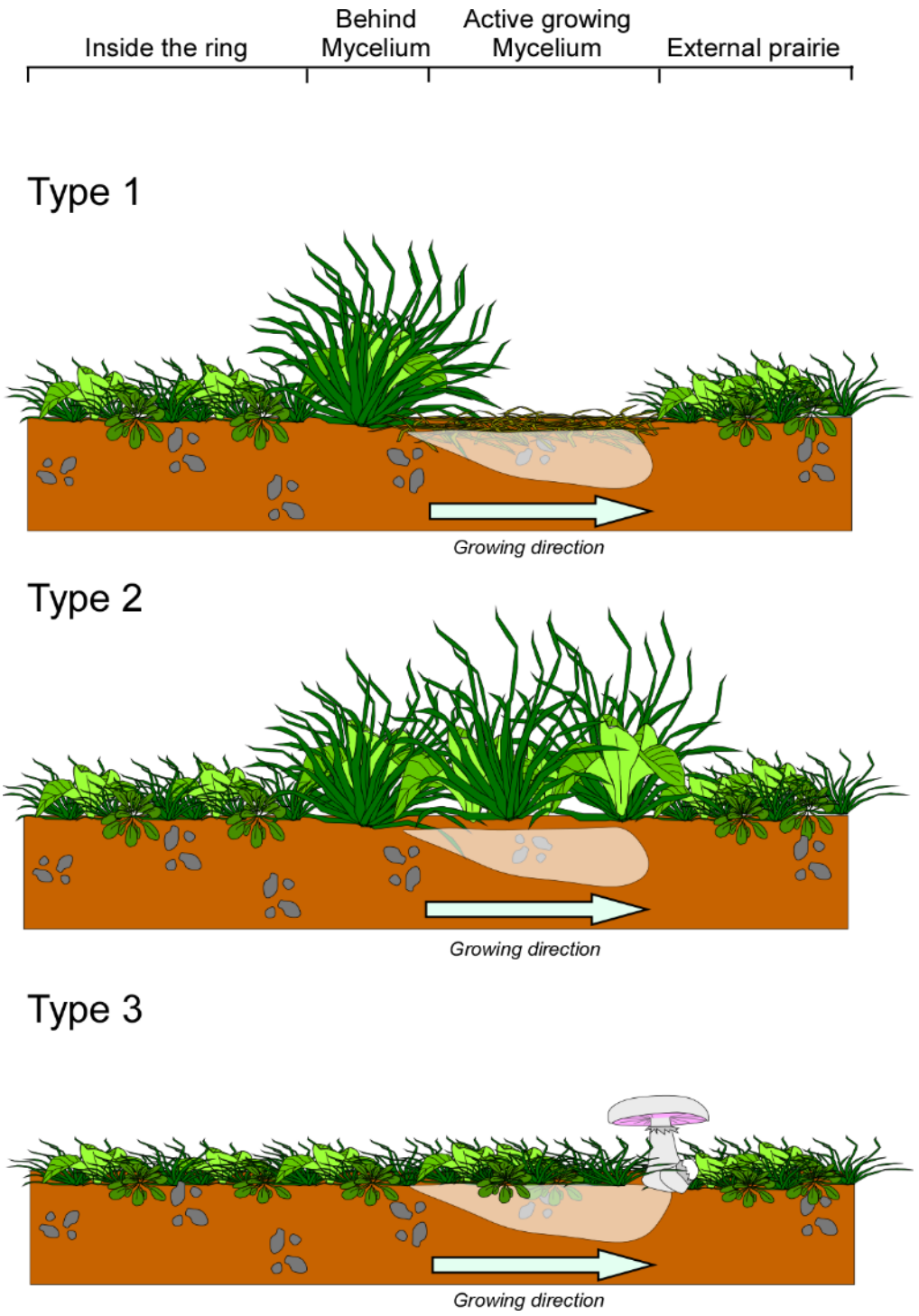

Figure 4.1.1 Diagram showing horizontal section of Type 1 (upper), Type2 (middle) and Type 3 FR (lower).

The ordination of grassland FRs is actually the most extensively adopted in works focusing on the effect of these fungi in natural or anthropogenic grassland, despite few study cases demonstrates some exception in these configurations. For examples, Type I FRs of *Marasmius oreades,* in different study sites, are characterized by two zones of stimulated vegetations bordering the zone of barren soil both externally and internally to the FR (Elliott, 1926; Hardwick & Heard, 1978). The same observation was anteriorly made in *Agaricus tabularis* FRs, although the outer stimulated zone appeared only in correspondence of carpophores

emergence period (Shantz & Piemeisel, 1917). Additionally, Edwards in 1984 and in 1988 conduced its studies analyzing the effect of a FRs of *Agaricus arvensis* with three internal areas of stimulated vegetation.

Most general classification was provided by Gregory (1982), that through a short bibliographic collection separates FRs in those formed by free living saprotrophs and ectomycorrhizal basidiomycetes beneath the name of free and tethered, respectively. The classification proposed was basically related to the trophic strategies of the fungi, whether plant symbionts of not. The observation proposed, indicated that the circular shapes assumed by the fungus is less related to the ecosystem in which FRs developed, but more probably to the specific ability of a FRs to expand in grassland and woodland in a constant circular way.

Finally, a more recent description FRs are classified according to configuration at different soil depth (Couch in 1995 and later by Fidanza in 2007). Both the authors classify FRs whether mycelial configuration in soil is associated to grass leaf litter or associated to deeper soil horizons, naming it leptophilic and edaphic. Following this rule, emerged that majority of the FRs in anthropogenic grassland (turfs and gardens) are leptophilic, meanwhile, FRs detected in natural or seminatural ecosystems are considered edaphic as they growing at deeper layers of soils. Outstanding examples of leptophilic FR forming fungus are *Vascellum curtisii, L. sordida*, and *Bovista dermoxanta* that grows at maximum depth of 4 cm and produces dense mycelium masses in correspondence of litter rich surfaces. Intriguingly, the different configuration of FRs fungi in soil horizon could be explained by the environmental condition in which the FRs develop. Then, the differential configuration could be attributable to management system of turfs and the oxygen requirement of the fungi (Hedges *et al.*, 2004). Indeed, although periodic watering provided in grassland decrease the dependency of the fungus from water, the adsorption of that water by soil may increase hypoxic conditions with soil depth. As consequence, the prohibitive condition in the deeper soil layers force fungi to growth on the surface. Oppositely, in natural and seminatural ecosystems less regular watering of soil promote formation of optimal aeration/humidity conditions for colonization of deeper layers of soils by fungal mycelium (Griffin, 1963; Miller *et al.*, 1989).

4.1.3. Spatial dynamics of FFR growth

Shapes of FRs

Usually, the expansion of fungal mycelium in soil respond to disparate stimulus (Carney & Matson, 2005; Gadd *et al.*, 2007). Main factor affecting fungal growth in natural environment were described and classified as :(a) abiotic and global, as large scale environmental variation i.e. precipitation and temperature (Moore *et al.*, 2008); (b) abiotic and local, at micro scale i.e. resources distribution (Ritz, 1995); (c) biotic and derived from other organisms i.e. competition for resources, parasitism, chemical interference (Dix, 2012); and (d) biotic and derived from the fungus itself i.e. self-regulation or auto-inhibition (Keller *et al.*, 2005).

Among these, one of the main factor regulating radial growth of mycelium and hyphal extension in fungi is the need to scavenge resources in unexplored spaces (Dix, 2012). This behavior is highly conserved in fungus with few exceptions for particular growth as such as formation of rhizomorphs and mold growth (Deacon, 2013). The centrifugal expansion of FRs appear to follow the same general rules whether considering

that the FRs expand in order to find soil organic matter in case of saprobic or ectomycorrhizal FR (Baldrian, 2008) and plant hosts, in case of pathogenic radial expansion (Shaw III, 1980; Pukkala *et al.*, 2005). In detail the centrifuge orientation of growth of FR is aimed to dislocate main fungal body from depleted resources area to a richer unexplored one. Therefore, in the newly colonized areas, fungal hyphae release enzymes act to degrade organic matter and reabsorb the scavenged resources (Šnajdr *et al.*, 2011; Rodrigues *et al.*, 2015). In the process of mycelial expansion, the mechanisms of advancement and release is continuously repeated in new spaces colonized and could be limitless if the rate of this process was not controlled by seasonal fluctuation of environmental conditions. Concomitantly, to the origination of new exploring mycelium, the internal areas of the FR became conditioned by the presence of senescent mycelium that impair backward colonization of the internal zones of the FRs. Particularly the negative feedback of the FR on himself is demonstrated by growth impairment of the vegetative mycelium whether soil sod containing the active mycelium is inverted as evidenced in *Clitocybe nebularis* woodland FRs (Dowson *et al.*, 1989). Following the combination of a need of new soil environments and the repulsion by older mycelium, FR originate this characteristic wave like expansion that in some case is described as donut-shaped.

Growth rates of FRs

Whether the formation of a ring is matter of the natural tendency of the fungus to escape and search unsuitable and suitable soils, the rate of expansion of the FR mycelium in ecosystem is dependent by different factors like seasonality, organic matter deposition and vegetation (Pugh, 1980). Additionally, evolutive fitness promoted divergent outcome in how a basidiomycetes fungus could growth rapidly or slower, or which are the stress-tolerance-optimum range according to the species-specificity and genotypic characteristics of the fungus. Indeed, higher variability is observed in studies describing growth rate of FRs from at interspecific and intraspecific level but also at ecosystem scale. Practically, Both FR ecologist and plant pathologists estimate annual growth rate of FRs in grassland, woodlands and gardens by tagging the external edge of the zones in correspondence of the active mycelium and monitoring the metrical advancement following a period of 1 year or during the vegetative season. The actual knowledge on FR expansion rates described it for nine different species. Among these, the highest values of growth were recorded for *Lepista sordida* in turfs with an annual growth of 125 cm (Terashima *et al.*, 2004). Subsequently, other species with a slower growth was *C. nebularis* as litter saprotrophs in woodland (90 cm) (Dowson *et al.*, 1989) and *A. arvensis* and *Agaricus campestris* in managed grasslands (60 cm) (Edwards, 1984; Bonanomi *et al.*, 2012). *Marasmius oreades* was largely described growing in a wide range between 7 cm/ yr to 49.5 cm/yr (Cosby, 1960; Burnett & Evans, 1966; Ingold, 1974; Hardwick & Heard, 1978; Dickinson, 1979). More interestingly, the fungus showing the slower growth range is *Tricholoma matsutake* being the only FR fungus belonging to the ECM group and woodland environment, accounting several reports indicating a growth between 6 and 17 cm per year (Ohara & Hamada, 1967; Lian *et al.*, 2006; Kataoka *et al.*, 2012; Narimatsu *et al.*, 2015). The same methodology allow to make an estimation of FRs ages when comparing diameter and growth rates, as in the case of FR of *Leucopaxillus giganteus* in French grassland, with an estimated age of 700 years and a diameter of 600 m (Gregory, 1982).

FRs shapes

The most characteristic aspect of a FR is the regular disposition of the mycelium at constant distance from the point in which the fungus originated. Plausibly, may be thought that each FR originate from a single point in soil and expands the circular configuration. At this rate, the source point of a FR could be interpreted as a newly germinated spore or a single fragment of mycelium expanding in a vegetative way.

Parker-Rhodes was the first scientist approaching to the genesis of the circular shape of the FRs and proposed to visualize the progress of the fungus as such as the waves generated by a drop of water in a pond. The parallel was suggestive because give the possibility to axiomatize that the ring-like shape is maintained in absence of microenvironmental discontinuities or perturbations. Hence, the hypothetic expansion of the fungus in soils with changing gradients of perturbations and resources can results in modification of the circular aspect of the FR shapes. Just to lists some of that factors, gradients of organic matters, water availability, concentration of specific chemicals in soil, parasitosis, autotoxicity and interspecific or intraspecific competition among FRs may promote delays or accelerations of mycelium. When the strength of negative factor impair mycelial growth the pattern of FR growth can magnify in the death and disappearance of a portion of the mycelium and the formation of non-circular FR.

These kinds of Formation are considered as Non-circular FRs and are listed as arcs, ribbons, mustaches or wings, spirals and rotors (Shantz & Piemeisel, 1917; Parker-Rhodes, 1955; Stevenson & Thompson, 1976; Karst *et al.*, 2016)(Figure 4.1.2). The comprehension of how these kinds of shapes are generated is still obscure, since formation of non-ring-shaped pattern require long-terms observations and extensive soil surveys. Partial understanding of the phenomenon is from literature approaching to the phenomenon through the use of mathematical modelling, rather than direct observation. Among mathematicians interested in the study of FRs kinetics and shapes Parker -Rhodes in 1955 treated theoretically the phenomenon. The author approach to the problem applying a simple geometrical model determined mainly by the proportion of ground occupied by the fungi, the age distribution of the individuals and the factors affecting interspecific competition among FRs. The model describes the phenomenon using two fundamental parameters, the dynamic of the mycelia in a uniform (not-bounded) field A; these parameters represent the growth of the mycelia at a constant rate ρ and the newborn rate σ. The mycelium is then described as a 2D cone, in which its vertex represents the time and place of birth of the ring.

On the basis of some geometrical considerations and probabilistic analysis, the FR will expand, grow, ageing and eventually encounter another FR. Additionally the model assesses the factors affecting interspecific competition among fungi, namely what happens when two fairy rings of two different species collide. The probability of two fungi to encounter is calculated according to their ages, the distance among their vertices and their radius. Furthermore, at the intersecting region among fungi three different possible events are considered. In the indifference case, both rings will continue to grow and therefore will coexist. In unilateral extinction only one is obliterated; the species eliminated could also not be reaching equilibrium again, and its occupation of the ground will depend on the newborn rate σ. Finally, in the bilateral extinction case they both get eliminated at the site of intersection but eventually, and this is the main result of the paper, the one with

larger $\rho^2\sigma$ would exclude the other. This factor $\rho^2\sigma$ has to be considered the factor of survival in competing FRs.

Nevertheless, in 1976 Stevenson and Thompson realized that the Parker-Rhodes model was only reliable for juvenile rings, therefore in small non-bounded areas and in a short time span. They then extended the model by including boundary effects and long-term behaviors. Therefore, the model is based on the same geometrical and probabilistic considerations, but the region occupied by the ring changes accordingly to boundary effects. Consequently, equilibrium in this system is reached in two different ways: either assuming that the ring would die by aging (therefore when reaching a certain size) or when approaching the boundary (mainly suitable in small fields). Therefore, they formulated two different equations for the average fraction of ground occupied by the fungi depending on the size of the field and found that the factor regulating interspecific competition was $\rho^2\sigma$ for small scales, in accordance with Parker-Rhodes (1955), but instead it was σ/ρ in large scales.

Finally, the last and more recent article uses a completely different mathematical approach karst (Karst *et al.*, 2016). In fact, instead of using geometrical and probabilistic assumptions, reaction-diffusion equations, representative of a pattern forming dynamic, are applied. The aim of this work is to qualitatively assess and reproduce the typical shapes of fairy rings, the most common round shape, but also isolated spirals and rotors. In doing so, fungal communities are described as a spatially distributed continuum of biomass (Davidson *et al.*, 1996b; Davidson *et al.*, 1996a), expressed by the variable b_t, growing and diffusing accordingly to the available resources r_t.

The model is then described by two coupled partial differential equation:

$$r_t = D_r \Delta_r - c_1 r b^2 + g(r_{max} - r)$$
$$b_t = D_b \Delta_b - c_2 r b^2 - mb$$

in which the parameters have the following connotation: the D's are the diffusion coefficients for the Laplacian Δ, m determines the mortality of the fungus and g is the replenishment rate with saturated level g_{max}; finally the crb^2 term represents the resource uptake by the fungi, in which c_1 is the rate of absorption of the resources by the fungus and c_2 is the efficiency of converting these resources into biomass.

Then numerical simulations are run by varying the mortality rate (m) and the substrate replenishment (g), since of experimental interest and therefore possibly useful for validation. Diffusion of the resources is assumed to be of the same order of the diffusion of water and furthermore the relation $D_r/D_b=2$ holds, meaning that resources diffuse twice faster than the biomass. Finally, in to achieve the different desired shapes, simulations are performed on a squared surface with two different initial conditions: a line source and a multiple point source. While the multiple source is more representative of the natural conditions, a line source is examined since from a mathematical perspective spirals are formed at the free end of a line, as well as from the interactions of multiple circular fronts. By investigating the parameter space, mainly through bifurcation analysis, it is then possible to achieve all the typical structures of FRs. Nevertheless, from the simulation they all

occur at the same ratio, whereas in nature rings are much more common than spirals or rotors. Two are the possible explanations: one is that the line source is less probable in nature, and the second is that it is unusual that when two rings collide, they will form a spiral, and this is also suggested by the simulation (in fact it requires high m and r). By these considerations the parameter space can be reduced in order to achieve a more realistic scenario in accordance to what observed in a natural environment.

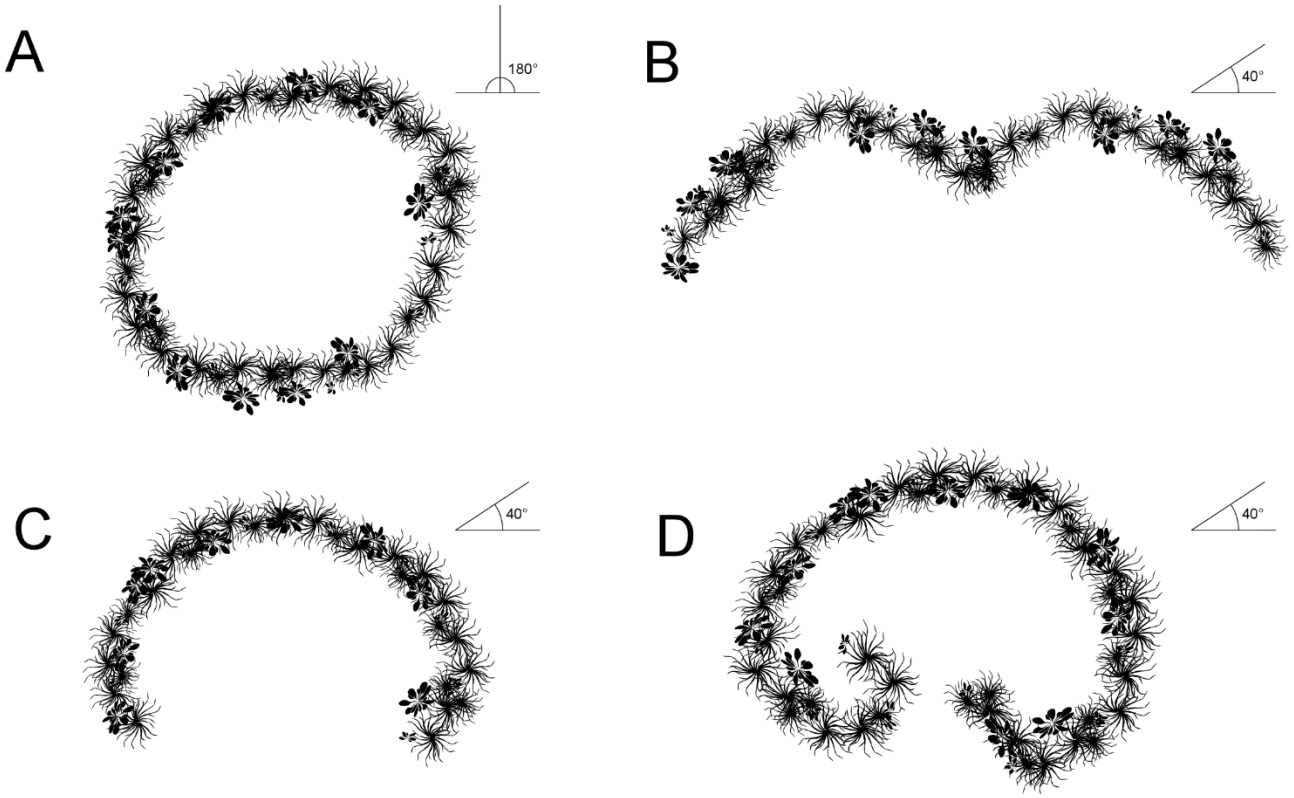

Figure 4.1.2 Diagram showing possible shapes of FR: Circle(A), wing, mustache or zig-zag (B), Arc (C) and rotor (D).

Mathematical modelling of the FR dynamics opens the way for a prognostic approach. The simplest methods to imagine the fragmentation of FRs is when considering disappearance of a mycelial portion by some detrimental factors developed in soils (Figure 4.1.3). The former product of the interaction is an interrupted circle or more generally an arc. With development of FR from arcs quite a few of possible outcomes may take place. In a condition in which the negative factor producing the arcs persists this could lead to FR disappearance, rather than, in case of a momentaneous detrimental cue the reformation of the ring can take place or a formation of characteristics forms of rotors. As previously stated, the fragmentation the growth of the mycelium restart in a multidirectional way with the potential to originate a new FRs or a portion of that. Indeed, the rotor shapes of a FRs is explained by the formation of new smaller colony of the fungus at the terminal tips of a FR arc pursuing free colonizable soil. The magnification of the phenomenon brings in some case to more complicated patterns in which smaller FRs are symmetrically nested in a progenitor one. On the other hand, in absence of disturbance or in constant microenvironmental conditions, the expansion of rotor, as also each form of non-circular FRs, can results in a restored circular distribution of mycelium after some years of growth explaining why is more common to find circular FRs that non circular one. Although the combination of factors controlling partial growth and death of FR mycelium and growth in time appear informative, explanation of

ribbons like and mustaches like forms arise in the case of involvement of intraspecific interactions among FRs. Several authors describe reciprocal obliteration of two or more FFRs (Shantz & Piemeisel, 1917; Parker-Rhodes, 1955; Ingold, 1974). In this case is still not clear whether intraspecific interaction can lead to out-competition of one of the interacting FR, but is probable, that following fungus development across different vegetative seasons the final results is a composite FR formed by more than one fungus of the same species (Murata *et al.*, 2005).

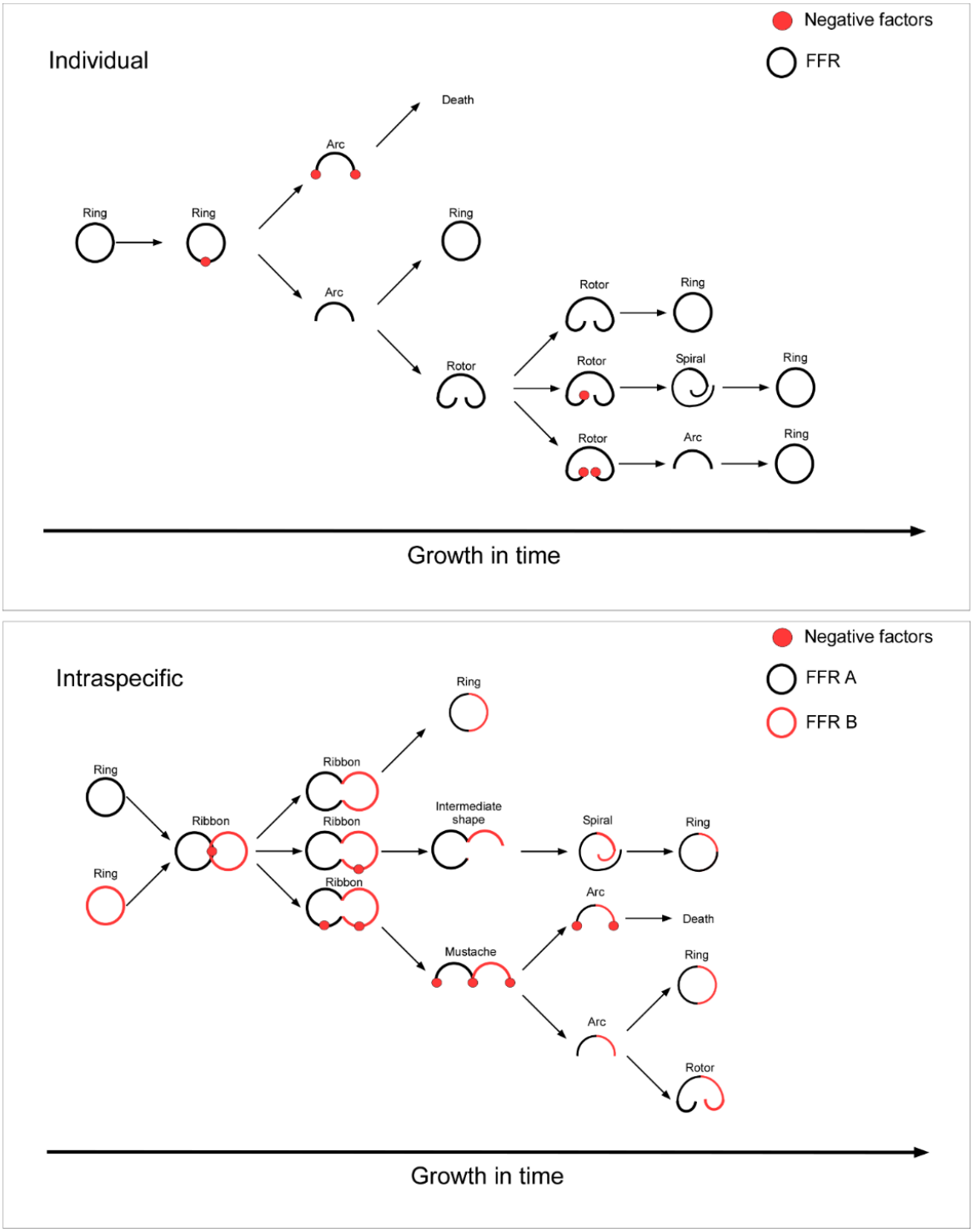

Figure 4.1.3 Two possible scenario of FR fragmented pattern development. Upper panel: individual development of fragmented pattern. Lower panel: fragmented patterns developed by intraspecific competition.

FRs in Basidiomycetes and why

As described in the previous sections, the process of expansion of mycelium is preserved in mycelia-forming fungi despite the wide variety of species and genera and the intrinsic functional variability of these (Deacon, 2013). So far, is plausible to think that each mycelia-forming fungus are able to create a FR. In an ideal condition this appears to be true, as in pure laboratory culture where continuous resources are available and biotic/abiotic interactions are extremely simplified. Inversely, in soil, patchy distribution of resources and complexity of interactions impose a filter for the formation of FRs. Only a limited portion of fungi were listed as forming FRs in natural environments. To date, majority of work on FRs, describe it as characteristics formation caused by Basidiomycetes fungi, with few cases for Ascomycetes fungi as *Tuber* and *Morchella* (Table 4.1.1)

Table 4.1.1 list of FR fungus described in present literature with name reported, current name and trophic strategy.

Phylum	Genus	Name reported	Current name	Trophic strategies	Reference
Basidiomycota	*Agaricus*	*Agaricus arvensis*	*Agaricus arvensis*	Sph	Halinsky and peterson (1970)
		Agaricus arvensis		Sph	Miller (2011)
		Agaricus arvensis		Sph	Atikinson (1900)
		Psaliota arvensis		Sph	Lees (1869)
		Agaricus arvensis		Sph	Ballion (1906)
		Agaricus arvensis		Sph	Edwards (1984)
		Abaricus arvensis		Sph	Edwards (1988)
		Agaricus camipestris	*Agaricus campestris*	Sph	Halinsky and peterson (1970)
		Agaricus campestris		Sph	Wollaston (1807)
		Psaliota arvensis		Sph	Jorden (1862)
		Agaricus campestris		Sph	Shantz & Piezemel (1917)
		Agaricus campestris		Sph	Van Thiegen (1884)

		Agaricus campestris		Sph	Ritzema Bos (1901)
		Agaricus campestris		Sph	Massert (1910)
		Agaricus campestris		Sph	Bonanomi (2012)
		Agaricus campestris		Sph	Miller (2011)
		Agaricus campestris		Sph	Xu (2011)
		Agaricus gennadii	*Agaricus gennadii*	Sph	Yang (2018)
		Agaricus lilaceps	*Agaricus lilaceps*	Sph	The Can Caesar-TonThat (2013)
		Agaricus sp.	*Agaricus sp.*	Sph	Olivier (1891)
		Agaricus tabularis	*Agaricus tabularis*	Sph	Shantz & Piezemel (1917)
	Agrocybe	*Agrocybe aegerita*	*Agrocybe aegerita*	Sph	Miller (2011)
	Amanita	*Amanita muscaria*	*Amanita muscaria*	Sym	Ludwig (1906)
		Amanita phalloides	*Amanita phalloides*	Sym	Ludwig (1906)
	Arachnion	*Arachnion album*	*Arachnion album*	Sph	Miller (2012)
	Boletus	*Boletus cavie*	*Boletus cavipes*	Sym	Ludwig (1906)
		Boletus elegans	*Boletus elegnas*	Sym	Ludwig (1906)
	Bovista	*Lycoperdon bovista*	*Bovista plumbea*	Sph	Wollaston (1807)
		Bovista dermoxantha	*Bovista dermoxantha*	Sph	Miller (2011)
		Bovista dermoxantha		Sph	Miller (2012)
		Bovista dermoxantha		Sph	Terashima (2004)
	Calocybe	*Tricholoma cgambosum*	*Calocybe gambosa*	Sph	Way (1847)
		Calocybe gambosa		Sph	Present study

		Agaricus prunulus		Sph	Evershed (1884)
		Calocybe camgosa		Sph	Guminska (1976)
	Calvatia	*Calvatia cyathiformis*	*Calvatia cyathiformis*	Sph	Shantz & Piezemel (1917)
		Calvatia cyathiformis	*Calvatia cyathiformis*	Sph	Halinsky and peterson (1970)
		Calvatia fragilis	*Calvatia fragilis*	Sph	Shantz & Piezemel (1917)
		calvatia polygonia	*calvatia polygonia*	Sph	Shantz & Piezemel (1917)
	Cantharellus	*Cantharellus cibarius*	*Cantarellus cibarius*	Sym	Lees (1869)
		Cantharellus cinereus	*Cantharellus cinereus*	Sph	Ludwig (1906)
	Catastoma	*Catastoma subterraneum*	*Catastoma subterraneum*	Sph	Shantz & Piezemel (1917)
	Chlorophyllum	*Lepiota morganii*	*Chlorophyllum molybdites*	Sph	Wiliams (1897)
		Lepiota morganii	*Chlorophyllum molybdites*	Sph	Shantz & Piezemel (1917)
		Lepiota morganii	*Chlorophyllum molybdites*	Sph	Stratton (----)
	Clavaria	*Clavaria sp.*	*Clavaria sp.*	Sph	Munch (1914)
	Clitocybe	*Agaricus geotropus*	*Clitocybe geotropa*	Sph	Lees (1869)
		Agaricus maximus	*Clitocybe maxima*	Sph	Munch (1914)
		Clitocybe caespitosa	*Clitocybe caespitosa*	Sph	Halinsky and peterson (1970)
		Clitocybe dealbata	*Clitocybe dealbata*	Sph	Halinsky and peterson (1970)

		Clitocybe dealbata		Sph	Guminska (1976)
		Clitocybe expallens	*Clitocybe expallens*	Sph	Guminska (1976)
		Clitocybe infundibuliformis	*Clitocybe gibba*	Sph	Lees (1869)
		Clitocybe nebularis	*Clitocybe nebularis*	Sph	Hearst (2013)
		Clitocibe nebularis		Sph	Dowson (1988)
		Lepista nebularis		Sph	Stahl (1900)
	Collybia sp.	*Collybia sp.*	*Collybia sp.*	Sph	Munch (1914)
	Coprinus	*Coprinus kubickae*	*Coprinus kubickae*	Sph	Miller (2011)
		Coprinus comatus	*Coprinus comatus*	Sph	Miller (2011)
		Coprinus sp	*Coprinus sp*	Sph	Miller (2012)
	Cortinarius	*Inoloma traganum*	*Cortinarius amethystinus*	Sym	Ludwig (1906)
		Telomonia armillata	*Cortinarius armillatus*	Sym	Ludwig (1906)
	Cuphophyllus	*Hygrophorus virgineus*	*Cuphophyllus virgineus*	Sph	Lees (1869)
	Cystoderma	*Cystoderma omiontinum*	*Cystoderma omiontinum*	Sph	Guminska (1976)
	Floccularia	*Floccularia luteovirens*	*Floccularia luteovirens*	Sym	Xing (2017)
	Gymnopus	*Collybia confluens*	*Gymnopus confluens*	Sph	Lees (1869)
		Marasmius urens	*Gymnopus peronatus*	Sph	Lees (1869)
		Gymnopus erythropus	*Gymnopus erythropus*	Sph	Falandysz (2013)
	Hebeloma	*Agaricus crustuliniformis*	*Hebeloma crustuliniforme*	Sym	Lees (1869)
	Hydnellum	*Hydnellum suaveolens*	*Hydnellum suaveolens*	Sym	Thanos (1905)
		Hydnellum suaveolens		Sym	Coulter et al (1911)

	Hydnum	*Hydnum compactum*	*Hydnum compactum*	Sym	Ludwig (1906)
		Hydnum repandum	*Hydnum repandum*	Sym	Lees (1869)
		Hydnum repandum		Sym	Ballion (1906)
	Hygrophoropsis	*Cantharellus aurantiacus*	*Hygrophoropsis aurantiacus*	Sph	Ludwig (1906)
	Inocybe	*inocybe sp.*	*inocybe sp.*	Sym	Wiliams (1897)
	Lactarius	*Lactarius insulsus*	*Lactarius insulsus*	Sym	Ludwig (1906)
		Lactarius torminosus	*Lactarius torminosus*	Sym	Ludwig (1906)
		Lactarius semisanguifluus	*Lactarius semisanguifluus*	Sym	Guminska (1976)
	Lactifluus	*Lactarius piperatus*	*Lactifluus piperatus*	Sym	Lees (1869)
	Lepiota	*Lepiota naiucina*	*Lepiota naiucina*	Sph	Halinsky and peterson (1970)
	Lepista	*Lepista nuda*	*Lepista nuda*	Sph	Miller (2011)
		Lepista nuda		Sph	Guminska (1976)
		Lepista sordida	*Lepista sordida*	Sph	Miller (2011)
		Lepista sordida		Sph	Terashima (2007)
		Lepista sordida		Sph	Choi et al (2010a)
	Leucopaxillus	*Clitocybe gigantea*	*Leucopaxillus giganteus*	Sph	Bayliss (1911)
		Clitocybe gigantea		Sph	Jorden (1862)
		Clitocybe gigantea		Sph	Van Thiegen (1884)
		Clitocybe gigantea		Sph	Ritzema Bos (1901)
		Clitocybe gigantea		Sph	Lees (1869)
		Clitocibe gigantea		Sph	Halinsky and peterson (1970)

	Lycoperdon	*Lycoperdon gemmatum*	*Lycoperdon perlatum*	Sph	Ballion (1906)
		Lycoperdon ciclicum	*Lycoperdon cyclicum*	Sph	McAlpine (1898)
		Lycoperdon wrightii	*Lycoperdon wrightii*	Sph	Shantz & Piezemel (1917)
		Lycoperdon marginatum	*Lycoperdon marginatum*	Sph	Miller (2011)
		Lycoperdon sp.	*Lycoperdon sp.*	Sph	Elliot (2002)
	Macrolepiota	*Agaricus pro-ceris*	*Macrolepiota procera*	Sph	Wollaston (1807)
		Macrolepiota procera		Sph	Guminska (1976)
		Macrolepiota mestoidea	*Macrolepiota mestoidea*	Sph	Guminska (1976)
	Marasmius	*Marasmius dryophilus*	*Marasmius dryophilus*	Sph	Falandysz (2013)
		Marasmius graminum	*Marasmius graminum*	Sph	Miller (2011)
		Marasmius oreades	*Marasmius oreades*	Sph	Abesha (2017)
		Marasmius oreadum		Sph	Evershed (1884)
		Marasmius oreades		Sph	Caspar (2015)
		Marasmius oreades		Sph	Blenis (2004)
		Marasmius oreades		Sph	Burnett & ev-ans (1966)
		Marasmius oreades		Sph	Fidanza (2007)
		Marasmius oreades		Sph	Fisher (1976)
		Marasmius oreades		Sph	Grams (2004)
		Marasmius oreades		Sph	Hardwick (1978)
		Marasmius oreades		Sph	Ingold (1974)
		Marasmius oreades		Sph	Mallet (1987)

	Marasmius oreades		Sph	Lawes, Gilbert and Worington (1883)
	Marasmius oreades		Sph	Williams (1887)
	Marasmius oreades		Sph	Caville (1898)
	Marasmius oreades		Sph	Massart (1910)
	Marasmius oreades		Sph	Bayliss (1911)
	Marasmius oreades		Sph	Moliard (1910)
	Marasmius oreades		Sph	Ballion (1906)
	Marasmius oreades		Sph	Withering (1796)
	Marasmius oreades		Sph	Reed (1910)
	Marasmius oreades		Sph	Wollaston (1807)
	Marasmius oreades		Sph	Jorden (1862)
	Marasmius oreades		Sph	Buckman (1870)
	Marasmius oreades		Sph	Van Thiegen (1884)
	Marasmius oreades		Sph	Ritzema Bos (1901)
	Marasmius oreades		Sph	Ludwig (1906)
	Marasmius oreades		Sph	Lees (1869)
	Marasmius oreades		Sph	Halinsky and peterson (1970)
	Marasmius oreades		Sph	Miller (2011)
	Marasmius oreades		Sph	Guminska (1976)
	Marasmius siccus	*Marasmius siccus*	Sph	Miller (2011)
	Marasmius sp.	*Marasmius sp.*	Sph	Miller (2012)

	Melanoleuca	*Tricholoma malaleuca*	*Melanoleuca malaleuca*	Sph	Shantz & Piezemel (1917)
	Paneolats	*Paneolats retirugis*	*Paneolats retirugis*	Sph	Halinsky and peterson (1970)
	Parasola	*Parasola pliicatilis*	*Parasola pliicatilis*	Sph	Miller (2011)
	Paxillus	*Paxillus involutus*	*Paxillus involutus*	Sym	Ludwig (1906)
	Pluteus	*Pluteus cervinus*	*Pluteus cervinus*	Sph	Charles(unknown)
	Psilocybe	*Psilocybe foenisecii*	*Psilocybe foenisecii*	Sph	Halinsky and peterson (1970)
	Suillus	*Boletus bovinus*	*Suillus bovinus*	Sym	Ballion (1906)
		Boletus variegatus	*Suillus variegatus*	Sym	Ludwig (1906)
	Tricholoma	*Tricholoma columbella*	*Tricholoma columbetta*	Sym	Massart (1910)
		Tricholoma equestre	*Tricholoma equestre*	Sym	Charles(unknown)
		Tricholoma grammopodium	*Tricholoma grammopodium*	Sym	Lees (1869)
		Agaricus bicolor	*Tricholoma personatum*	Sym	Munch (1914)
		Agaricus bicolor		Sym	Buckman (1870)
		Agaricus bicolor		Sym	Lees (1869)
		Tricholoma sp.	*Tricholoma sp.*	Sym	Williams (1887)
		Agaricus terreus	*Tricholoma terreum*	Sym	Wollaston (1807)
		Tricholoma matzutake	*Tricholoma matzutake*	Sym	Ohara (1967)
		Tricholoma matzutake		Sym	Kim (2013)
		Tricholoma matzutake		Sym	Kim (2014)
		Tricholoma matzutake		Sym	Narimatsu (2015)

		Tricholoma matzutake		Sym	Li (2016)
		Tricholoma matzutake		Sym	Oh (2016)
		Tricholoma matzutake		Sym	Katooka et al. (2012)
		Tricholoma matzutake		Sym	Lian (2006)
	Unknown	*Sterile basidiomycete*	*Sterile basidiomycete*	Sph	Miller (2011)
	Vascellum	*Vascellum curtisii*	*Vascellum curtisii*	Sph	Miller (2012)
				Sph	Terashima (2004)
				Sph	Miller (2011)
	Armilaria	*Armillaria mellea*	*Armillaria mellea*	Pat	Present study
	Heterobasidion	*Heterobasidion annosum*	*Heterobasidion annosum*	Pat	Present study
Ascomycota	*Morchella*	*Morchella esculenta*	*Morchella esculenta*	Sph	Ballion (1906)
		Morchella hybrida	*Morchella hybrida*	Sph	Ballion (1906)
	Tuber	*Tuber sp.*	*Tuber sp.*	Sym	Tulasne & Tulasne (1851)

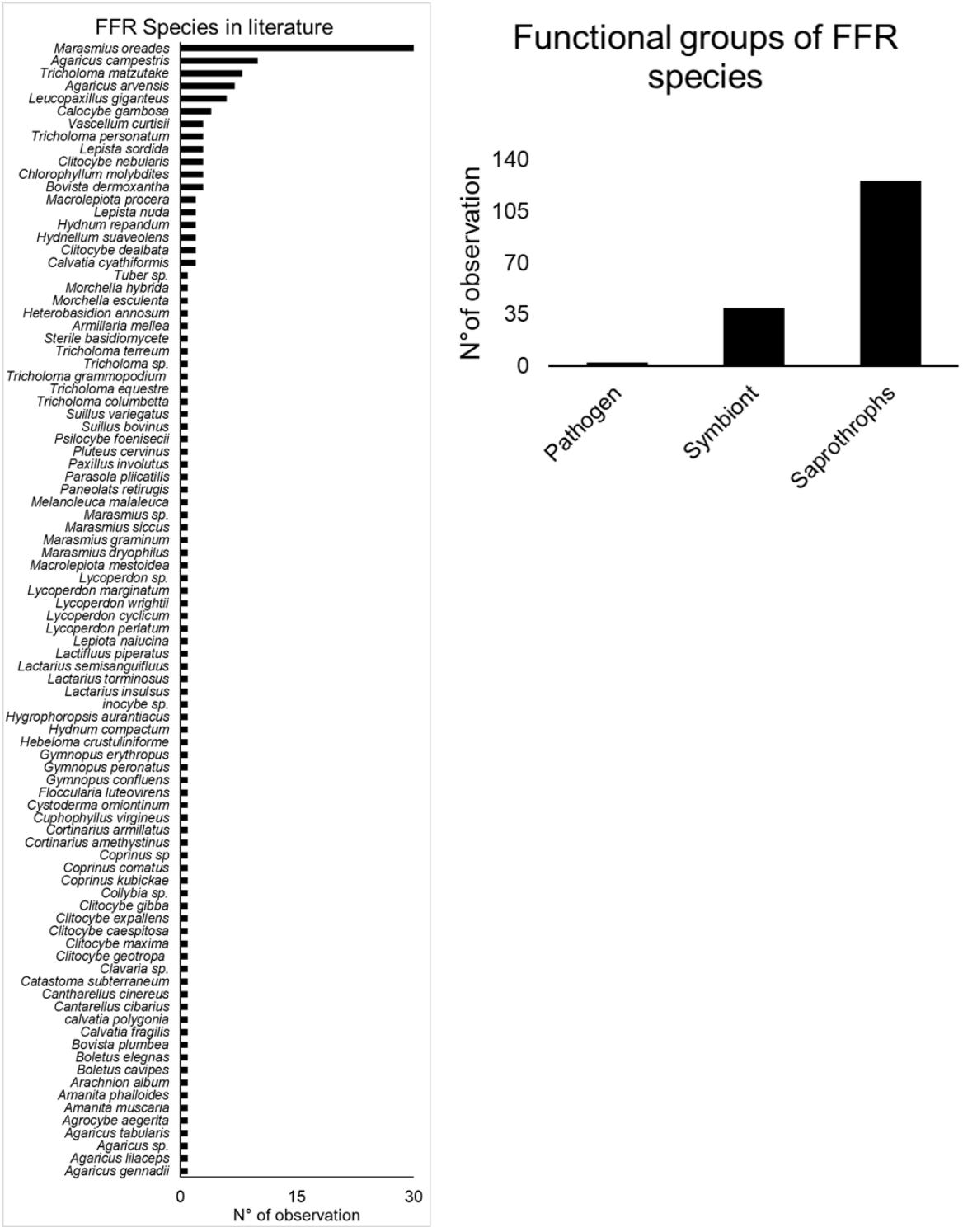

Figure 4.1.4 Panel on the left, showed the most studied species and more frequent records of FR forming fungal species. Panel on the right show the number of recorded FR classified according to fungal trophic strategies.

The ability to form FRs restricted to Basidiomycetes phylum find an exhaustive explanation via particular adaptation of the phylum. Several authors describe most of Basidiomycetes as C-selected species compared to other phyla that are mainly composed by R-selected and S-selected taxa (Hiscox *et al.*, 2018). The selective strategies for fungi suggests that C-selected species are characterized by slow growth rate, ability to adapt to diverse trophic resources and combative behavior with respect to competitors (Dix, 2012; Boddy &

Hiscox, 2016). In physiological terms, the description of the C-selected strategies among fungi means that the species are able to defend itself and its resources through the synthesis of complex molecules. Additionally, the C-selected strategy overcome the disadvantages from the dependence of a single trophic resources because the development of a richer arsenal of degrading enzymes (Baldrian, 2008; Spiteller, 2008). On the other side, the high energetic costs of this evolutive development leads Basidiomycetes to growth with slower replicative rates but with the possibility to find a proper response to disparate environmental conditions. In light of this, environmental conditions as such as patchy distribution of resources, competition and predation by other microbial species are less effective on Basidiomycetes allowing him to growth in a regular pattern (Warcup, 1959; Nishino *et al.*, 2017). This kind of strategic adaptation is well described in organic matter decomposition successional patterns, which with ageing is dominated by Basidiomycetes that became able to persist on it until the exhaustion of a resource and exclude other microbial taxa in its active zone (Frankland, 1998; Osono & Takeda, 2001a). This, traduced in terms of FRs formation, indicate that decreased responsiveness to potential interfering agents for fungal growth lead Basidiomycetes to be the fungal phylum more able to form FRs.

4.1.4. Abiotic and biotic interactions of FFRs

Abiotic interactions

In its expansion pattern FRs markedly changes soil abiotic properties in all the ecosystem where it was studied. Works reporting physicochemical changes in soil from FRs development are concentrated on grassland ecosystems, while woodland FRs, given the dependence to fruiting body emergence to detect it, has been scrutinized only in few cases (Kim *et al.*, 2013).

Majority of works addressed to elucidate FR effects approaching the problem visualizing the change promoted by the fungus before its outward migration, in unaffected soils (OUT), in soils with presence of vegetative mycelium (ON) and soil that experienced the passage of the fungus (IN). The approach, clearly explain the alteration of the abiotic parameters of soil but lead to misunderstanding when considering the different typology of FRs. Hence, whether the OUT and IN zones commonly describe the constitutive abiotic characteristics of the grassland exteriorly and interiorly to the FR, the analysis of the ON zones may describe different kinds of soil conditioning action from the FR as it is that ON zone can correspond to the area in which is observed a detrimental effect of the fungus on grass cover or a positive one.

However, as general consideration, the process of FRs soil conditioning is variable among ecosystems, vegetative state of the fungus, species of basidiomycetes and, of course, depend by the pedological characteristics of the soil affected. The main effect shared among the different type of FRs are: i) in the active zone FRs produce a decrease of organic matter and Ph because as effect of their saprobic activities; ii) The hyphal development of FRs increase soil hydrophobicity. iii) An enrichment of nutrient is observed in soils affected by the FR fungus.

The first statement of the extent to which FR affect soil characteristics was provided by Shantz and Pietzmel that collected and compared data from previous works with that from a multiseasonal survey in Colorado grasslands. In that cases emerged that in soils experiencing FR passage was not present a significant

change in total Nitrogen values but, was instead observed an increase in the contents of primary plant nutrients as such as Ammonium and Nitrates. They also provides first evidence that the process was commonly find in different FR species because recorded in *A. tabularis* and *C. cyathiformis.* Similar results to those from the Colorado FRs demonstrated chemical changes imposed by *M. oreades* in perennial grassland, pastures and turfs. Also in that cases Ammonium and Nitrates was the nutrients majorly enriched in the ON areas of the FRs (Elliott, 1926; Norstadt *et al.*, 1973; Fisher, 1977; Hardwick & Heard, 1978). In parallel to the increase of Ammonia and Nitrates, *M. oreades* FRs also showed variable increase of phosphorous, in a range between 20% and 100 % respect the soil external to the FRs and a coupled depletion of Potassium for values around 50%. The way in which FRs changes in soil chemistry appear conserved among species but showed variation according to the developmental state of the fungus forming it. For example, the comparison of soil nutrients of the same FR of *M. oreades* prior to fruiting and at the beginning of sporophores production reveals that with sporophores appearance the nutrients previously enriched in soil disappears. Concomitantly with nutrient depletion in soil increased level of Ammonium, Nitrates and Phosphorous were found in carpophores indicating translocation of resources (Fisher, 1977). The displacement of nutrients from the vegetative area of the FR towards the carpophores of the FR fungus lead to the hypotheses that these nutrients were more probably immobilized in the mycelium instead to be freely distributed in soil. Accordingly, to the suggestion proposed, studies on *A. arvensis* FRs with three concentric zones of changing vegetation showed that mycelium advancement produce significant variation in Total Nitrogen, available Phosphorous, Potassium and Magnesium in soil but not find a correspondent enrichment of Total Phosphorous in plants that showed simulated growth. The different level of available Phosphorous in soil and Total Phosphorous in plants indicates that the supposed enrichment was more probably an effect of nutrients immobilization (Edwards 1988, 1984).

Despite, this is still not demonstrated whether the FRs are promoter or suppressors of mobilization of nutrient from the organic matter decomposed. Despite this, research effort continued to move in the direction to find a relation between nutrient budget and effect on vegetation. The effect of *M. oreades* FRs in European grassland was investigated as modifier of nutrient available for plant growth. Soil descriptions indicates specific enrichment of Nitrogen compounds as organic Nitrogen, Ammonium, Nitrate and Proteins in the *M. oreades* influence zone. Specular effect was assessed for other macronutrients as such as Calcium, Potassium, Phosphorus, and Magnesium (Gramss,2005). Later, in perennial grassland of North America, effect on chemical and physical characteristics of soils from *A. campestris* development was scrutinized. Consistently with the general effect previously observed from FR development, a marked enrichment of Ammonium-Nitrogen, Phosphorous and Sulphur was detected within the ON zone, while no significant changes were assessed for other chemicals. From the observation arise the hypothesis that the exceeding enrichment of Ammonium-nitrogen compounds, coupled with increasing of hydrophobicity and production of toxic compounds was the triggering factor promoting plant death in turfgrasses (Fidanza, 2007). The same fungus, in a species-rich Mediterranean grassland soils, preferentially accumulate Ammonium instead of Nitrates in early spring, while, shift form negative to positive variation of Nitrates in summer. Similarly, with what was detected for Nitrates, the quantity of Phosphorous available was accumulated in the early vegetative season reaching values doubled

compared to soil of the external grassland, and enriched at lower level in July (Bonanomi *et al.*, 2012). Similarly, Type II *Agaricus liliaceps* FR from North American grassland exert enrichment of Ammonium, Nitrates and Phosphorous in the zone correspondent to vegetative mycelium (Caesar *et al.*, 2013). More recently works on FRs has been concentrated in Asian grassland from Tibetan plateau. Xing and co-workers in 2017, tried to identify ECM helper bacteria from a grassland saprotroph, despite the unusual attempt, they describe an enrichment of Phosphorous in soils correspondent to *Floccularia luteovirens* mycelium. More properly, Yang in several works on FRs of Asiatic grasslands, attempted to explain the appearance of plant stimulated zone and find enrichment of nutrients in correspondence of it. More interestingly, from works on *Agaricus gennadii* in Tibetan plateau is stated that the effect of nutrients enrichment decreases with the advancement of the vegetative season probably following the vegetative state and activity of the fungus. Specifically, an increase of available Phosphorous in fungal active zone was registered, with respect to total Phosphorous, total Nitrogen and total inorganic Nitrogen that showed generally a decrease of the values when shifting from the OUT to IN through the ON zone (Figures 4.1.4 and 4.1.5).

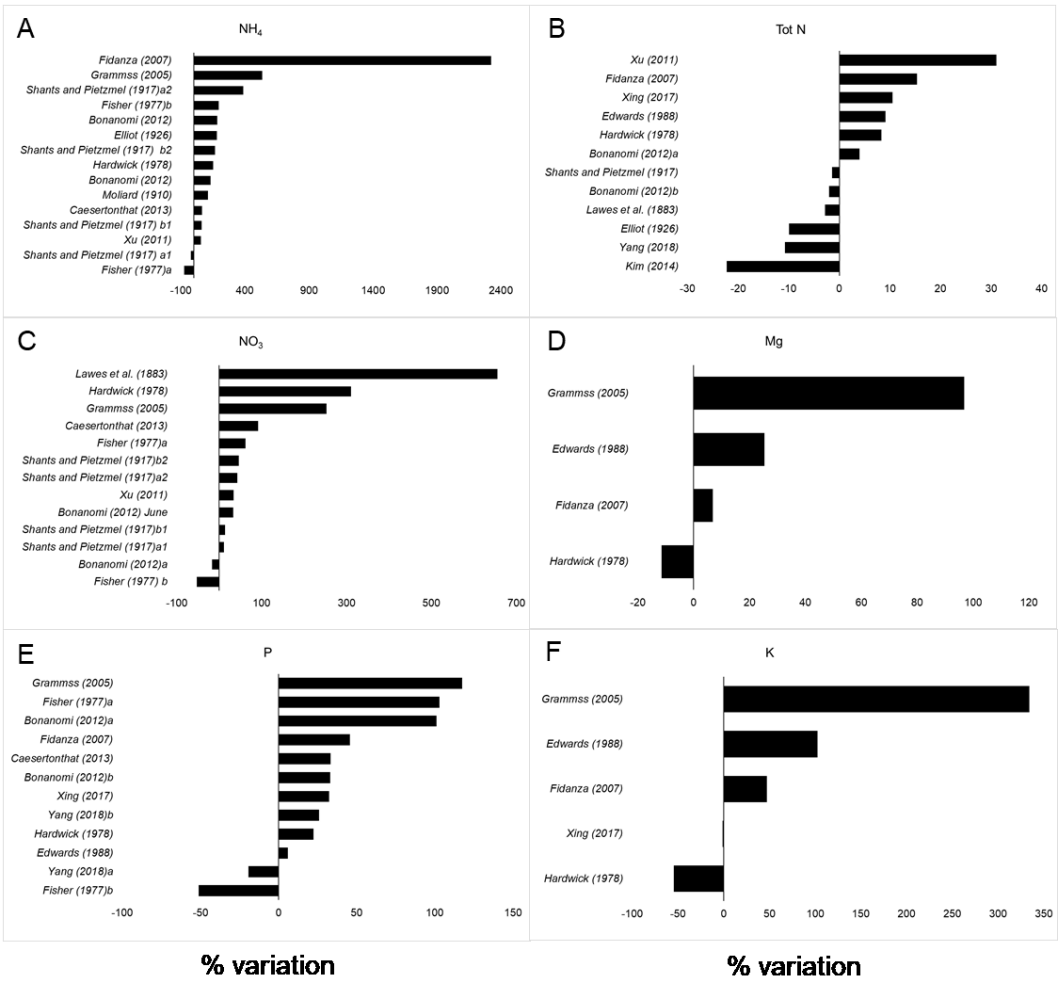

Figure 4.1.4 Bibliographic collections of studies reporting macronutrient changes promoted by FR passage. Data are reported a percentage variation in Ammonia (A), Total N (B), Nitrates (C), Magnesium (D), Phosphorus (E) and Potassium from soils external to FR and soils in correspondence of FR forming fungus fungal fronts.

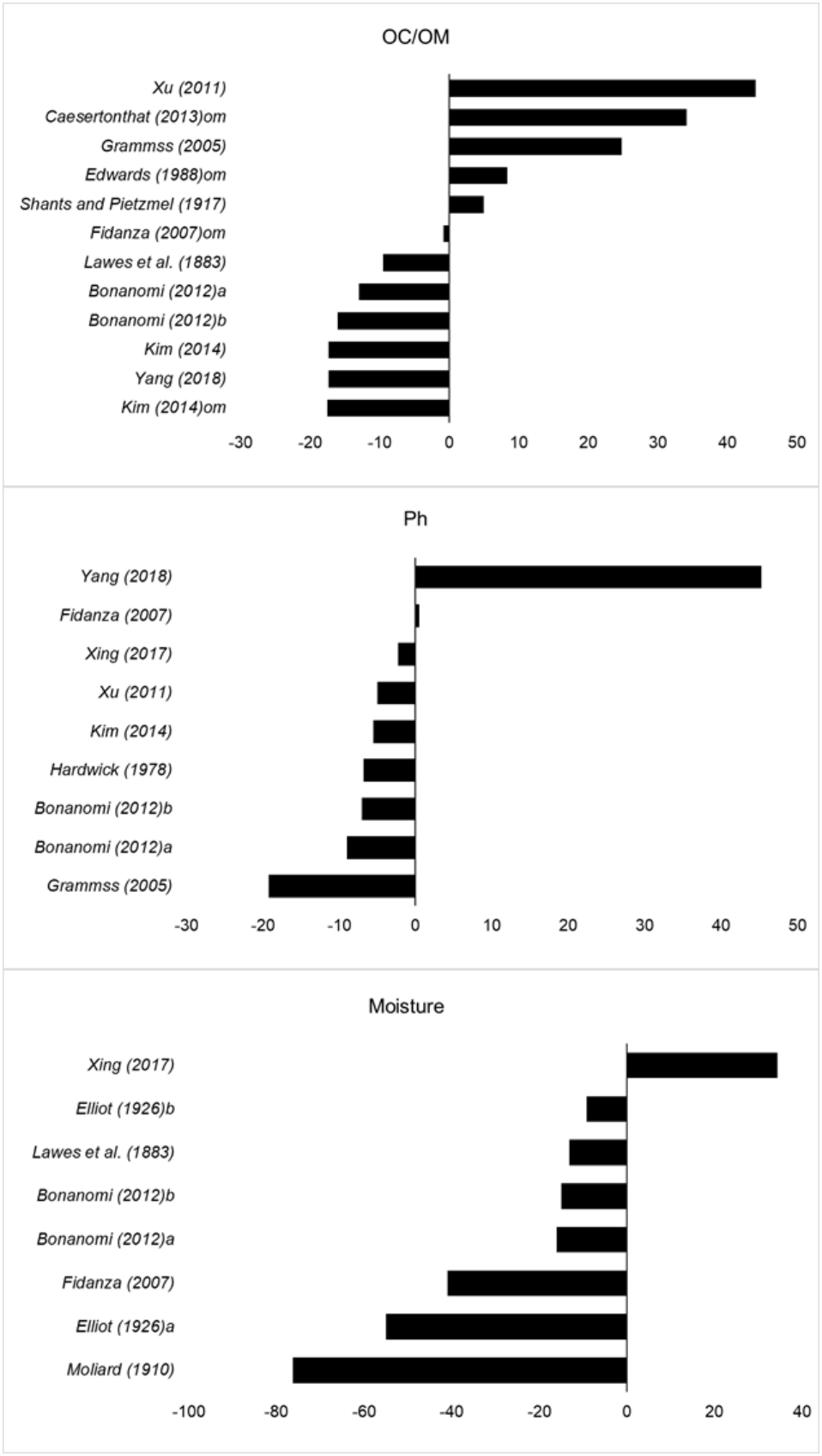

Figure 4.1.5 Bibliographic collections of studies reporting macronutrient changes promoted by FR passage. Data are reported a percentage variation in Organic matter/Organic carbon (upper), Ph (middle) and Moisture (down) from soils external to FR and soils in correspondence of FR forming fungus fungal fronts.

Biotic interactions

Plants

Fairy rings in grassland ecosystems differently affect extant vegetation. The Type I was the most studied because of its combined effect as inhibitor and promoter of plant growth. The effect of Type I FRs development was in some case undesired, as it creates discontinuous grass coverage in gardens, lawns, golf turf and crops (Fidanza, 2007). In these cases, both the instauration of a zone with a necrotic vegetation and the appearance of a stimulated one was considered as negative, leading to a research effort aimed to find the more effective methods for FRs eradication (Miller *et al.*, 2012). As consequence, different authors tested application of different fungicides or verified the effect of an opportune mineral fertilization for controlling FR-forming fungus (Smith, 1980; Blenis *et al.*, 1997; Elliott *et al.*, 2002). This argumentation was extensively studied in a field of phytopathology that was mostly aimed to furnish helpful information to solve the problem rather than understand the mechanism behind the vegetation decline. However, those study increase the knowledge concerning importance of FRs in ecology.

Several hypotheses were formulated about the deadly effect of the active mycelium on plants in Type I FRs. Shantz and Piezemel proposed the appearance of zone with dead vegetation was an effect of the severe drought taking places in soils experiencing the growth of the mycelium. The effect was attributed to the significative release of hydrophobins in soils (Sietsma *et al.*, 1995), that, when excavated, appeared whitish and with a floccular consistence given the massive mycelium occupation. Their observation was supported by multiseasonal records in which they consider with a lesser extent of the effect in years with regular and abundant precipitation, rather than the effect is more appreciable in dry seasons. A comparative evidence supporting the theory was presented in the same work. Indeed, the authors do not constrain their observation to a single FR-forming fungus but include in their observation the Type II fairy ring forming fungus *C. cyathiformis.* Indeed, from a visual observation of the soil experiencing the development of type II *C. cyathiformis* FRs a scarce occupation of the mycelium was detected when compared to those of the Type I FR of *A. tabularis.* The finding supported the idea that mycelial induced hydrophobicity is directly correlated to the detrimental effect of the fungus in Type I FRs. However, the observations on Colorado grassland FR do not considered the findings in studies of Elliott that, before and later the review of Shantz and Piezemel, provided evidence of the phytotoxicity from watery extract of soil affected by Type I FRs of *M. oreades.* The extracts applied to various grass seedlings modifying root architecture and producing deterioration of fine roots of plants (Elliott, 1926). Successive studies identify that Type I FR forming-fungus has potential effect to produce phytotoxic compounds as such as cyanides (Blenis *et al.*, 2004). The process was furtherly emphasized by (Caspar & Spiteller) in 2015 that propose the production of cyanuric based compounds is conserved in nature among different kingdom but fungi are more able to use it as defensive agent storing hydrocyanic acid in the form of free cyanohydrin. Additionally, Caspar and co-workers suggest that the described mechanism is conserved among different Type I FR forming fungi. However, the authors cited as cyanogenic fungus also those belonging to the *Clitocybe* genus that are most commonly Type III and do not shows, to the best of our knowledge, detrimental effect on vegetation. Furthermore, the model fungus for FRs studies *M. oreades* was repeatedly

described to be Type I or Type II because differentially showed the presence or absence of pathogenic behavior. In turn, ambiguous effect of *M. oreades* leading to a certain level of uncertainty regarding the effectiveness of detrimental chemicals as unique factor promoting the formation of a Type I FR. The evidence of the impairment of nutrient uptake in FFR soil from extant plants was afforded by Edwards in 1984 and 1988 from a Type I FFR of *A. arvensis* in English grassland. The authors observed in grasses the presence of anomalous coloration on leaf tips as common symptoms of nutrient deficiencies in plants. In its surveys Edwards compared the level of nutrients available in soil and those presents in plant tissues concluding that the effect was produced by the deficiency in Phosphorus that, even if highly enriched in soils corresponding to FR active mycelium, was not correspondently enriched in the leaves of *Agrostis capillaris.* The finding leads to the hypothesis that the limitation in phosphorous availability was caused by the immobilization of the nutrient internally to the mycelium of the FR fungus. Conceivably with Edwards, the hypotheses connected with the nutrient uptake impairments was supported by the work of Gramms in 2005 that described unbalanced levels of nutrients between root and shoots in grasses subject to the passage of *M. oreades* FR. More recently another work scrutinizing changes in chemical composition of soil affected by *A. campestris* in USA support the idea that the detrimental effect of the FR fungus could be dependent by toxic over-enrichment of nutrients and the production of harmful sulfuric compounds (Fidanza, 2007).

Taking in account the effective ecological importance of a Type I FR, several authors reported that the harmful effect of FR, despite no principal causative agent has been identified, produce a considerable effect on plant communities in grassland. Generally, in all the works analyzing these effects the results assessed that the extant dominants plant species in the grassland analyzed undergo a more or less severe decline that permits the instauration of a community with a different hierarchical structure in the zones early experiencing the passage of the fungus or where the active mycelium of the FR is still present. The effect is commonly described as a temporary effect because plant grassland community restore at the same organization of the outside grassland in the inner zone of the ring. Shantz and Piezemel noticed association between Type I FRs and the increase in abundance of broad leaves species in the internal zones of the FR while no community changes was detected in Type II FRs. The authors explain that the harmful effect of the fungus trigger the instauration of a natural rotation among grassland plant species. With more detail, Cosby in 1959 reported data of a plant community changes between the external grassland the area where vegetation is stimulated inside the FR and the more internal zone of the FR. Interestingly the work analyzed the plant community on three FRs Type I FRs of *M. oreades* in USA grassland with an increasing gradient of grazing regimes. The results showed a more marked changes in plant community operated by the FR fungus in area with lower grazing regimes compared to moderately or heavily grazed areas. The effect of Type I *M. oreades* FRs in UK pastures on plants yields and community structure was similarly described by Hardwick in 1978. The authors described the effect of the fungus with the intent to find a managing practices because of the undesired effect of the FR fungus halving productivity of pastures of 50 % and producing low quality hay for livestock. Interestingly, he found that following the zone of bare vegetation appears in dry season and is quickly recolonized with following fall precipitations by stoloniferous and weed plant species. Later in 1986 Edwards observed the same community-

shift effect in its tripartite Type I FR of *A. arvensis* in Southern England stating it as factor changing natural plant organization without detailed explanation of the community changes. With more detail, the effect of *A. campestris* Type I FRs on plant community organization of a Mediterranean grassland of central Italy was explained in a study by Bonanomi and coworkers in 2012. The authors collect vegetation data in four zones across the FR in the attempt to describe community structure before the passage of the FR in a zone named OUT, in the zones where active mycelium was present, called FF as acronym of Fungal front, the area of stimulated vegetation named Belt and a more internal site IN. Result of the works clearly identified coherence and repulsions of specific plant abundance to each zone of the FR transect and, moreover, identifies specific plant communities that thriving exclusively in the inner part of the ring increasing plant gamma diversity in Mediterranean grassland. In the same site Zotti (present document) studied Type I FR of *A. arvensis* with same methodologies and the addition of two sampling site internal to the FR to check at which point from the passage of the FR the original plant community of grassland is restored. Similarly, to the previous finding a deep change in community was observed, particularly with the abrupt instauration of short-lived, fast-growing plant species in the zones behind the vegetative mycelium of the fungus. In the work is suggested that the discontinuities created by the FR forming fungus decrease competitive ability of dominant species lead to a first colonization by opportunistic species. Moreover, in the same work, a parallel functional analysis of the bacterial community associated to the FR transect showed the presence of bacterial community selected for degradation of xenobiotic compounds in the zones with presence of active mycelium, supporting the hypothesis that the detrimental effect of the fungus on the plant community may be caused by the toxification of interested soils. In a general view when plant community composition changes was stressed as effect of FR in grassland, the common idea from different authors was that FR act as an agent of disturbance, that, as in the case of Rogedano grassland, promoting maintenance of species richness in conjunction of other agent as, hail, snow, fire, grazing allelopathy and phytotoxicity. Indeed, Bonanomi and Zotti propose Type I FRs as ecosystem engineer in calcareous grassland.

By the point of view of the stimulant effect of FR on vegetation, a certain level of scientific effort was produced to detangle the mechanisms. As usual, the first hypotheses regarding the functioning area with stimulated vegetation was produced by Shantz and Piezemel and particularly from the results of their chemical surveys in both type I and Type II FR. They suggested that the stimulated growth of plants is from the ammonia released by the FR fungus that in turn is transformed in nitrates by extant bacterial community becoming available for plants, hypotheses also supported by other authors (Cosby, 1960; Edwards, 1988) and still adopted in more recent work focused on nutrients availability(Xu *et al.*, 2011; Yang *et al.*, 2018c; Yang *et al.*, 2019). However, the deeper investigation of the conditioning effect of FR on soil nutrient release weakens the nutrient dependent hypothesis as it was advanced the idea that those nutrients was immobilized into the mycelial body of the FR fungus (Fisher, 1977). Other author does not exclude the nutrient release hypothesis but explain the phenomenon as is may be additionally caused by the structuration of a favorable microbiome supporting plant growth in the stimulated belt of a FRs (Bonanomi *et al.*, 2012; Caesar *et al.*, 2013). Indeed, evidence of the structuration of a favorable community was provided by Zotti that through a metagenomic

screening find microbial actors as the genus *Trichoderma* and *Burkholderia* and fungal guilds as Arbuscular mycorrhizal fungi with acknowledged beneficial effect on plant protection and growth in correspondence of soil with the presence of the FR fungus and in the stimulated strips of vegetation.

Aside the theories of nutrient enrichment and formation of positive soil microbiome, a parallel topic explained the formation of area with stimulated vegetation through the emission of chemicals promoting plant growth. Actually, these compounds are known as "fairy chemicals" and was for the first time described from a pure culture of *L. sordida,* a common pathogenic basidiomycete in Asiatic gardens forming type I FRs (Choi, J-H *et al.*, 2010; Choi, JH *et al.*, 2010; Mitchinson, 2014). From a fine process of fractionation Choi and colleagues tested the growth-regulatory effect of the extract on bentgrass seedlings, leading to the isolation of the active compound 2-azahypoxanthine (AHX). The compound enhanced root and shoot elongation of tested plants with optimal effect at a concentration of 20 µm. Most interestingly, *L. sordida* extract induce changes in gene regulation of plant exposed promoting the up-regulation of Glutation-S-trasferase (*GST*), aquaporines (*OsTIP;1*) and Bowman-Birk type proteinase inhibitors (*BBI*) genes that in turns suggest an increased ability of plants in nitrogen nutrient up-takes, resistance to saline stress, detoxification from xenobiotics and resistance to pathogens. Moreover, application of the extracts from the FR fungus in culture of tea cells demonstrating a dose dependent effect supporting cellular proliferation in a similar fashion to other basic hormones (Kawagishi, 2019). Coupled with AHX form *L. sordida* extract imidazole-4-carboxamide (ICA) was isolated and tested in turfgrass and rice seedlings showing a plant-growth regulator property. In detail ICA inhibit growth of tested plants suggesting that the compounds may be consider responsible of the growth impairment characteristic of the Type I FR instead of the development of stimulated bands of vegetation (Choi *et al.*, 2017). However, the application of the compound on adult plants increased rise grain yields of 26 % indicating variable effect on plant growth and productivity. More interestingly ICA and AHX shares the same precursor, 5-aminoimidazole-4-carboxamide (AICA). The finding of promising molecules applicable in agriculture supported investigation efforts towards the description of metabolic pathway by which plant became able to use AHX growth regulator (Choi *et al.*, 2014). Description of the unedited pathway of plant hormone production identified that AICA derivate AHX became available for plant in form of 2-aza-8-oxohypoxanthin (AOH) and, promptly later, methodology for synthetic production of the compounds was developed as bio-stimulants for agriculture de-spite scarce yields from the method. In light of the low efficiency in synthetic synthesis of AOH the same author tried massive synthesis of the compounds through activity of bacteria belonging to *Burkholderia* genus using the resting cell methodology (Choi *et al.*, 2016). Among the *Burkholderia* species tested, the results indicated that *Burkholderia contaminans* was the most efficient in the process of bioconversion from AHX on AOH with a yield of 100% of transformation. More recently, investigation on fairy chemicals developed intri-guing direction in plant biochemistry fields as it is demonstrated the ability of plants to support endogenous production of these regulators from an unknown purine pathway even scrutinizing genetic pool of *L. sordida* (Takano et al., 2019). More detailed knowledge on the topic and processes of formation of fairy chemicals are reviewed in (Kawagishi, 2019).

Microbial community

Whether the effect of FR on plant community was a matter of study in grassland ecosystem the study of the effect of the fungus expansion on soil microbial community has been mostly investigated in woodland soils. Consistent research energy was redirect toward the study of the microbial community in the 'Shiro' (Japanese name of FRs) of *T. matsutake* because of the increasing commercial interest for Mycorrhizal Helper Bacteria (MHB) applicable for the development of the commercial exploitation of the basidiomycetes or forestation practice as ECM symbionts (Powell, 1993; Yun & Hall, 2004). The higher interest posed in studying woodlands microbiomes was also supported by the development of next generation sequencing that was earlier applied in those ecosystems and only later in grassland FR with pure ecological interests.

Among culture-based studies, a first exploration of the topic has been produced scrutinizing the effect of ECM *T. matsutake* 'Shiro' in Japanese *Pinus densiflora* forests. Through plate dilution method, samples of soil outside, on and inside the FR was assessed that FR impose a generalized simplification of the bacterial community (Ohara & Hamada, 1967). Inversely for what observed in woodland soil, no significant changes was observed in culturable fungi and bacteria of Colorado grasslands on five Type I *M. oreades* FR (Norstadt *et al.*, 1973). In detail, the study hypothesize that the high release of urea produced by the degradation of organic matter of the fairy rings affect microbial activity and magnitude. Inversely from expectations a sharp decrease in urease activity was observed in the active mycelial zone. Related to the unchanged magnitude of microbial population the evidences indicated that the microbial community was not able to enzymatically transform the high level of Urea and induced to paucity in the active zone of the FR. Moreover, in studied on *Agaricus liliaceps,* FAME profiling associated to DNA sequencing methodology reveals that in the rhizosphere of stimulated zone of the FR, bacterial community increase in diversity and became more present compared to the whole fungal population (Caesar *et al.*, 2013). Inversely, a decrease in microbial activity and biomass was also observed in the inner portion of *A. campestris* FR in Italian grassland (Bonanomi *et al.*, 2012).

Following these studies, mainly based on culture methodologies, the study of microbial communities associated to FR mycelium was monopolized by the application of metagenomic methodologies. Hence, back to woodland FRs, high throughput molecular methods was applied to describe a detailed effect of competition among ECM forming fungus. Structural changes in ECM community was observed following the development of *T. matsutake* FRs that lead to the simplification of the community expressed as decrease of ectomycorrhizal tips of *P. densiflora* (Lian *et al.*, 2006). The works was the first in describing a microbial community changes caused by FR using PCR-based methods with respect to a culture-based one, the results showed the possibility to obtain more precise and quick results from molecular tools. As consequence, more detailed results was obtained through PCR-DGGE when compared to plate dilution methods in assessing the effect of *T. matzutake* FR on the soil bacterial community. Concordantly with the previous work a simplification in bacterial community is observed in the active zone of the FR both in culture and molecular results but the last evidenced that the community simplification was not generalized within the bacterial community, because of few taxa persisting to the effect. In that case, *Sphingomonas* and *Actinobacterium,* persisting in the active fungal zones (Kataoka *et al.*, 2012). Regarding fungal community, the development of *T. matsutake* FRs in Korean forests

produced a similar simplification effect as observed from 454 pyrosequencing of DNA extracted outside, on and inside the "shiro". A significative decrease in fungal diversity is observed at the passage of *T. matsutake* in exception to changes in the equitability among the different taxa of the community that appear unaffected. However, the work maintained a generalized descriptive aspect lacking helpful multivariate approach for further understanding of metagenomic data (Kim *et al.*, 2013). The lack of a deeper exploratory methods was equally present in the subsequent work from the same research group studying prokaryotic community in soil affected by *T. matsutake* FRs. However, inversely to what observed for the fungal community, the bacterial community appear responsiveness to the passages of the fungus with no remarkable changes in diversity metrics applied (Kim *et al.*, 2014). In discordance to these pioneer work, opposite results were obtained from the same ecoregion that visualized together the changes in both eukaryotic and prokaryotic microbial community following development of *T. matsutake.* Works was aimed to describe microbial community structure in relation to identify microbial figures facilitating culture of *T. matsutake* as exploitable edible mushroom. From that survey no apparent changes were observed for the eukaryotic community, meanwhile specific changes and association was observed for the bacterial community of soil dominated by *T. matsutake.* In detail, the author analyzed with different metrics the change in microbial community compositions among different soil, Bray Curtis similarity for fungal community and Uni-Frac distance for the bacterial one. The results stated that geographic position was a better predictor of fungal community composition with respect to the effect of the passage of the FR fungus. Oppositely, non-significative association was observed for geography and bacterial community that appear more susceptible to the development of *T. matsutake*. From the FRs analyzed is evinced that some bacterial genus has a common advantageous response from community simplification triggered by the fungus. In particular *Burkholderia, Bacillus* and *Paenibacillus* genus (Oh *et al.*, 2016). With the same proposal, those genera and other identified in the study was tested as mycorrhizal helper bacteria in controlled condition. The study clearly assessed that majority of bacterial taxa accumulated in soil dominated by *T. matzutake*, including bacterial genus belonging to *Burkholderia*, has negative effects on FR fungus development while only two genera belonging to *Paenibacillus* and *Staphylococcus* support a significant growth of the fungus oh (Oh & Lim, 2018a).

In a more general view, further works described effect of Tibetan plateau FRs on microbial communities of soil, specifically bacterial, in the area of stimulated vegetation. The effect of *Agaricus gennadii* was tested in alpine and temperate climatic conditions (Yang *et al.*, 2018b). In both grassland the FR development showed a parallel trends increasing diversity indexes in the area corresponding to the stimulated vegetative belts leading to the formulation of an hypothesis in which the strong release of nutrients operated by the decomposing effect of the fungus produce an increased productivity of the prokaryotic community and the plant community in the study. Despite the suggestive plethora of parameters put in place in this study few detailed information was provided regarding changes and associations of specific bacterial taxa, although the authors declare to work on metagenomic data at taxonomic species level. More recently, the community structuration of microbiome associated to the development of *A. arvensis* showed that across the FR few significant changes are observed for the Number of observed taxonomical units, Shannon diversity index and Pilou's Evenness for

bacterial community while more significant changes was observed in the fungal community alpha diversity at the development of the FR fungus (Zotti 3.3 section). Despite the different response in species richness, a multivariate approach to the data demonstrated a dramatic change imposed by the fungus in both the community that was subsequently assessed being due to the specific increase of *Burkholderia* genus among bacterial taxa and *Trichoderma* among the fungal ones. The specific association of these microbial taxa was explained as a potential phenomenon of mycoparasitism. Indeed, supporting evidence from other bibliographic study on this phenomenon explained that those taxa persists to the simplification effect of the FR since *Burkholderia* showed the ability to convert harmful toxins produced by the fungus as ICA in AHX (Choi *et al.*, 2016) or *Trichoderma* has low sensibility to cyanide produced by *M. oreades* (Blenis *et al.*, 2004) ii) both genus showed inhibitory effect on the growth of the culture associated fungus (Oh & Lim, 2018a; Oh *et al.*, 2018) and iii) the microbial taxa has acknowledged application in biological control of fungal population in agricultural field (Kubicek *et al.*, 2001; Parke & Gurian-Sherman, 2001; Benítez *et al.*, 2004). These considerations also supported the speculation in which the persistence of these taxa may be described as an evolutive advantage developed to face defensive strategy of their elective parasitic host.

Table 4.2.2 Bibliographic collection and focus, of studies and species regarding FR from 1917 to nowadays.

Species	Type	Mechanisms			Focus	Site	Ref
		Community effects	Stimulated Bands	Necrotic Bands			
Agaricus tabularis	T1	Favour weeds	Nitrates	Hydrophobic soil	P	USA Grassland	(Shantz & Piemeisel, 1917)
Calvatia cyathiformis	T2	no changes	Nitrates	Nr	P	USA Grassland	(Shantz & Piemeisel, 1917)
Marasmius oreades	T1	Nr	Nr	Cyanuric compounds	P	USA Lab.	(Elliott, 1926)
Marasmius oreades	T1	Change of the community structure depending by the manging practices of the area.	Nitrates	Hydrophobic soil	P	USA Grasslands	(Cosby, 1960)
Marasmius oreades	T1	Nr	Nr	Hydrophobic soil	P		(Lebeau & Hawn, 1963)
Marasmius oreades	T1	Nr	Nr	Parasitism	P	USA Lab.	(Filer, 1965)
Tricholoma matsutake	Th	Disappearance of bacteria in the inner zone	Nr	simplification of the community.	B	Japan Woodland	(Ohara & Hamada, 1967)
Marasmius oreades	T1	Nr	Nr	Nutrient, Hydrophobic	P, B	USA	(Norstadt *et al.*, 1973)

Marasmius oreades	T1	Nr	Nr	Soil. Nutrient immobilization for plants	P	Canada Turfs	(Fisher, 1977)
Marasmius oreades	T1	First colonization operated by stoloniferous species and weed (annual).	Nr	Nr	P	UK Grassland	(Hardwick & Heard, 1978)
Agaricus arvensis	T1	changes plants organization	Nitrates	Hydrophobic Soil and Nutrient uptake impairments	P	UK Grassland	(Edwards, 1984; Edwards, 1988)
Marasmius oreades	T1	Nr	Nr	Hydrophobic Soil	P		(Ayer *et al.*, 1989)
Marasmius oreades	T1	Nr	Nr	Toxic compounds	P		(Sutton, 1990) 1989
Marasmius oreades	T1	Nr	Nr	Cyanide	P, F	Canada Turfs and Lab.	(Blenis *et al.*, 2004)
Vascellum curtisii	T1	Nr	Nr	Pathogenic behaviour	P	Japan Turfs and Lab.	(Terashima *et al.*, 2004)
Bovista dermoxanta	T1	Nr	Nr	Pathogenic behaviour	P	Japan Turfs and Lab.	(Terashima *et al.*, 2004)
Marasmius oreades	T1	Nr	Nr	Hydrophobic Soil and Nutrient uptake impairments	P		(Gramss *et al.*, 2005)
Tricholoma matsutake	Th	Exclusion of most ECM species in the ring	Nr	Nr	F	Japan Woodland	(Lian *et al.*, 2006)
Agaricus Campestris	T1	Nr	Nr	Over enrichment of NH_4 and H_2S.	P	USA Grassland	(Fidanza, 2007)
Lepista sordida	T1	Nr	2-azahypoxanthine (AHX)	Nr	P	Japan, Lab.	(Choi, J-H *et al.*, 2010)
Lepista sordida	T1	Nr	Nr	application of ICA as inhibithor of plant growth	P	Japan, Lab.	(Choi, JH *et al.*, 2010)

Agaricus campestris	T2	Nr	Shift toward different sources of nitrogen	Nr	P	China grassland	(Xu *et al.*, 2011)
Tricholoma matsutake	Th	disappearance of bacteria in the inner zone	Nr	Nr	B	Japan Woodland	(Kataoka *et al.*, 2012)
Agaricus campestris	T1	Favour short lived plants	Formation of Empty niche and beneficial microbial community	Hydrophobic soil/release of detrimental compounds	P, F, B	Italy Grassland and Lab.	(Bonanomi *et al.*, 2012)
Tricholoma matsutake	Th	TM became dominant and dramatically simplify the community	Nr	Nr	F	South Korea Woodland	(Kim *et al.*, 2013)
Clitocybe nebularis	Th	Nr	Nr	Inibithory effect against phytophtora	F	UK, Woodland	(Hearst *et al.*, 2013)
Agaricus liliaceps	T2	Nr	Nutrients/microbiome	Nr	P, B	USA Grassland	(Caesar *et al.*, 2013)
Tricholoma matsutake	Th	no difference in bacterial community	Nr	Nr	B	South Korea Woodland	(Kim *et al.*, 2014)
Tricholoma matsutake	Th	effect for bacteria and no effect for fungi	Nr	Nr	F, B	South Korea Woodland	(Oh *et al.*, 2016)
Tricholoma matsutake	Th	Nr	Nr	Nr	F	South Korea, Lab.	(Oh *et al.*, 2018)
Tricholoma matsutake	Th	Nr	Nr	Nr	B	South Korea, Lab.	(Oh & Lim, 2018a)
Floccularia luteovirens	nr	community simplification	Nr	Nr	F, B	China Grassland	(Xing *et al.*, 2018)
Agaricus gennadii	T2	no changes detected in the community	stimulation of plant biomass through decrease of N:P ratios	Nr	P	China Grassland	(Yang *et al.*, 2018a)
Agaricus gennadii	T2	no changes detected in the community	Sensitivity of FR plant to Q10 values	Nr	P	China Grassland	(Yang *et al.*, 2018c)
Agaricus gennadii	T2	Increase of bacterial diversity in the stimulated zone zones.	Stimulated plant biomass	Nr	P, B	China Grassland	(Yang *et al.*, 2018b)

Agaricus gennadii	T2	FR fungi are important factors in ecosystem functioning and stability in temperate and alpine grasslands	Stimulated plant biomass	Nr	P, B	China Grassland	(Yang *et al.*, 2019)
Agaricus campestris	nr	FR fungi are important factors in ecosystem functioning and stability in temperate and alpine grasslands	Stimulated plant biomass	Nr	P, B	China Grassland	(Yang *et al.*, 2019)
Agaricus arvensis	T1	Simplification of the communities and instauration of opportunistic species	Favourable microbiome for plant	Production of xenobiotics	P, F, B	Italy, Grassland	Zotti (subshapter 2.2)
Calocybe gambosa	T1	Simplification of the comunity	nr	Hydrophobicity	F, B	Spain, Botanical garden	Zotti (subshapter 2.3)

4.1.5. Concluding remarks

In our view the study of FRs offers an exceptional opportunity for scientists and even students to approach to soil ecology. This is mostly given by the fact that FRs offers visualization of a real model in which interactive effect of Basidiomycetes could be visualized (Figure 4.2.6). Moreover, FR studies include de possibility to connect different field of scientific research like microbiology, chemistry and botany. Detailed motivations evidencing the advantage of studying FRs was listed by (Norstadt *et al.*, 1973) latterly stating that:

(i) each fairy ring site is a replicate within a given species of grass and soil type;
(ii) soil properties are affected in a short distance;
(iii) the fungus lives for scores of years;
(iv) there are seasonal fluctuations in fungal activity;
(v) vegetation responses and soil effects enable locating of the fungus;
(vi) sod sections can be moved to the greenhouse or laboratory growth chamber for study;
(vii) the fungus can be isolated and cultivated in pure culture.

In some case the opportunity offered by the visualization of the Basidiomycetes expansion in field condition was exploited to collect important multidisciplinary information such as the genotypic composition of *M. oreades* population in Norwegian sand dunes (Abesha *et al.*, 2003) and the use of FR as a proxy of enhanced nitrogen enrichment provoked by climatic changes affecting uptake strategies of plant community in alpine Asiatic meadow (Yang *et al.*, 2018a).

In light of the relevance that we posed in FR studies some points still needs to be clarified. As first consideration we believe that classification applied by (Shantz & Piemeisel, 1917) remain the most informative for grassland FR, however the cases as such as those *M. oreades* and *A. arvensis* forms variable pattern of changing vegetation could be afforded extending the actual ordination with a subclassification.

Nevertheless, connect fungus specificity and effect on extant vegetation and ecosystem functioning is a tough challenge. This because:

1- FRs effect are variable depending rain budgets (drought favor formation of zone with death vegetation).
2- The direct dependence of the nutritive substrate deposed available for the fungus that changes according to plant productivity and therefore leaf litter quality (Bonanomi *et al.*, 2009), resources distribution (Carney & Matson, 2005), water availability, Ph and biogeography (Fierer, 2017).
3- Lack of extensive databases on fungal genomes making next generation sequencing techniques, like shot-gun sequencing but also non-functional metagenomic sequencing, less reliable compared to the prokaryotic counterpart in soil community; and even because the basic uncertainty of fungal genetics given their dikaryotic nature.

About mechanisms of action of FR in soil and how they modifies the surrounding environment, is still needed to understand which are soil parameter directly affected. Therefore, despite the extensive description of the soils conditioned by the development of FRs, is still not clear if the process of enrichment of the ON zones is mainly attributable to mycelium nutrient content or to free nutrients in soil. Also, the detrimental effect on plant and microbial communities by the release of toxic compounds could not be considered as singular factor strong enough to produce Type I FR symptoms on extant vegetation. This because of the lack of multi-specific and direct, literature evidence and because, reasonably, some authors describe the products of FR fungus metabolism like “Fairy Chemicals” as a promising plant growth enhancer with agricultural purpose. In light of this, the perspective of the cooccurrence of other factors as such as the increase of soil hydrophobicity or the deterioration of the physiological status of plants subject to the passage of the FR could be taken in consideration with more emphasis.

Another pivotal topic that need to be disentangled in order to understand the global importance of FRs in ecosystem is connected to FR growth rates and pattern of formation. Actually, is possible to oversee the importance of FR because of its effect but not possible to quantify the rate and the regularity in which a population or a community of FRs affect soils. Therefore, our description and discussion in the section reserved to the configuration of FR in soil and fragmentation pattern follow a *Hypothesis non fingo* because of the lack in knowledge. Moreover, explanation of factors promoting formations of non-circular/fragmented shapes in FRs are multiple and are the same reported in literature on Basidiomycetes but in scattered way. Given the increasing evidence suggesting FRs as ecosystem engineer, the need of a review work aimed to clarify the typology of the interactions that basidiomycetes should face in order to form a FR if actually pivotal in order to understand detail within ecosystem dynamics.

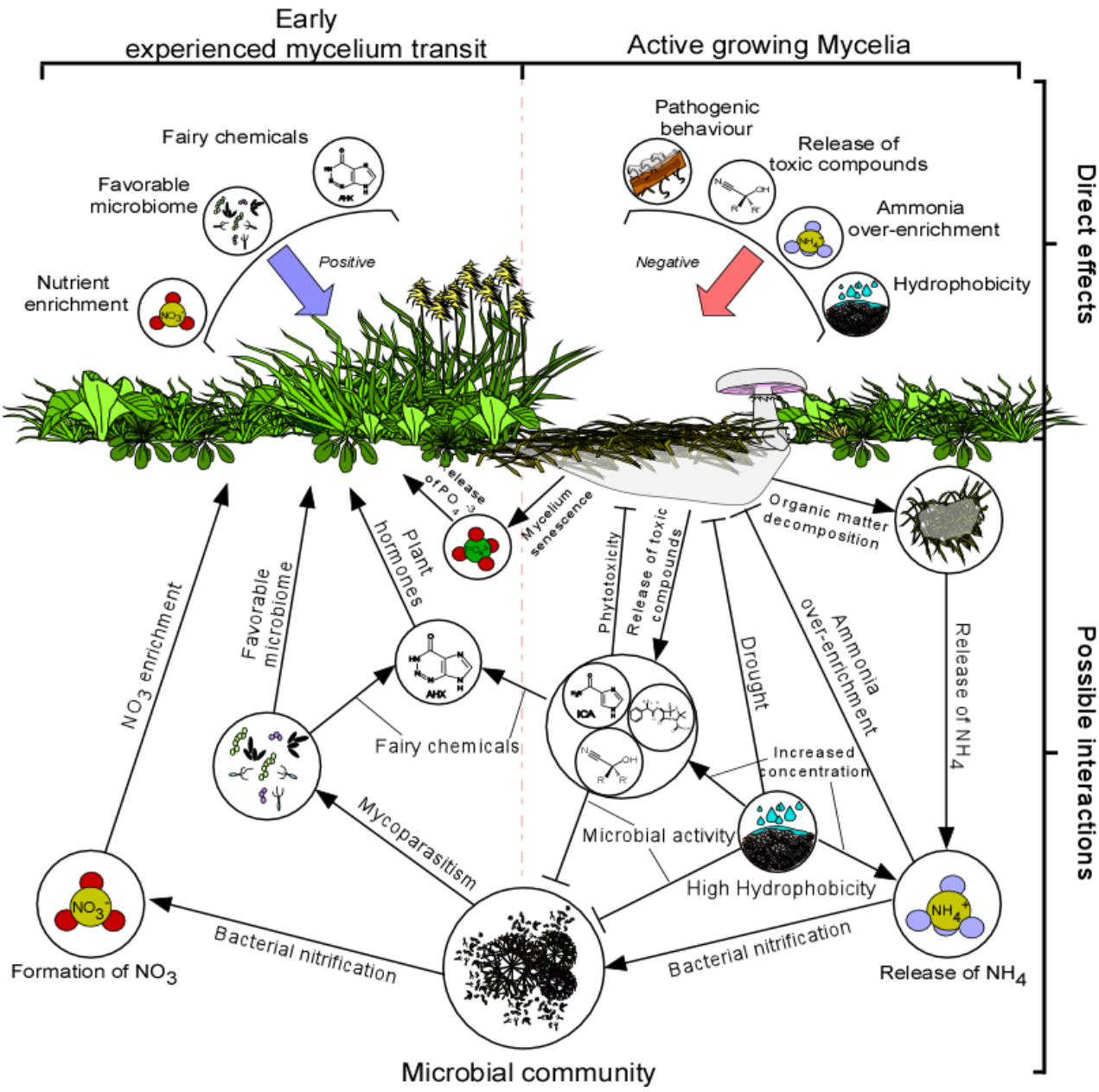

Figure 4.1.6 Soil Interaction described for FRs

4.2. One ring to rule them all: a killer fungus fosters plant and microbial diversity in grassland

4.2.1. Introduction

Grassland ecosystems are distributed in all of the world's ecoregions (Olson *et al.*, 2001; Hoekstra *et al.*, 2005). Among global ecosystems, temperate grasslands are considered biodiversity hotspots (Mittermeier *et al.*, 2011; Wilson *et al.*, 2012; Habel *et al.*, 2013), being anomalous given that species richness is generally higher towards the equator (Gaston, 2000). Indeed, in semi-natural Mediterranean grassland, species richness can challenge that of tropical forests (Dengler *et al.*, 2013; Habel *et al.*, 2013). The high level of species richness is maintained by, and strictly depends on, the development and mixed effect of environmental and biological factors such as grazing, fires, hail, frost, snow cover, water budgets, plant-plant competition, facilitation, phytotoxicity and autotoxicity (Belsky, 1992; Tilman & Downing, 1994; Grace, 1999; Callaway & Aschehoug, 2000; Bonanomi *et al.*, 2005; Mazzoleni *et al.*, 2007; Mazzoleni *et al.*, 2010; Rice, 2013; Vincenot *et al.*, 2017). Moreover, the relation between plant species distributions and the soil microbial community explaining ecosystem functionality has become a more central topic in recent years (Bezemer *et al.*, 2006; Van Der Heijden *et al.*, 2008). In light of this, the balance between fungal symbiont, pathogens and saprotrophs (Klironomos, 2002; Teste *et al.*, 2017; Semchenko *et al.*, 2018), or the extent to which the bacterial community decomposes plant material is crucial to realize the peculiarities of plant community patterns (Bartelt-Ryser *et al.*, 2005; Bonanomi *et al.*, 2019). Recently, Mazzoleni *et al.* (2015a) reported the discovery of the inhibitory effect of extracellular DNA of plant species, released by litter decomposition, on individuals of the same species. The same effect was also observed on several other taxa, including fungi and bacteria (Mazzoleni *et al.*, 2015c). This phenomenon has been suggested to be important for the formation of vegetation patterns, including plant rings (Cartenì *et al.*, 2012; Bonanomi *et al.*, 2014), and species coexistence (Cartenì *et al.*, 2016).

A specific case of a plant-soil interaction mediating species richness in grassland ecosystems are fungal fairy rings (FFRs) (Edwards, 1984; Bonanomi *et al.*, 2012; Caesar *et al.*, 2013). FFRs are concentric vegetation bands caused by the expansion of basidiomycetes fungi in soil which, in most cases, alter the existing plant community (Shantz & Piemeisel, 1917; Halisky & Peterson, 1970; Edwards, 1988). FFRs have been described as exerting a negative, positive or neutral effect or a combination of the above in plant communities. Hence, Shantz and Piezemel in 1917 classified FFRs into three types: a first (Type-1) formed by a bare area of dead vegetation found close to the underground-expanding fungal front and followed by a belt of luxuriant vegetation; a second (Type-2), clearly recognizable by the presence of luxuriant vegetation but without an external area with dead/poor vegetation, and a third (Type-3) where the ring is periodically revealed by the presence of fungal carpophores with no detectable changes in vegetation patterns.

Historically, greater attention has been attracted by Type-1 and Type-2 FFRs. Several attempts have been made to describe the decline of vegetation and the vegetative explosion following fungal development in FFRs. In some cases, FFRs were studied because they were considered agents of Phyto-pathological problems for turf management (Elliott *et al.*, 2002; Fidanza *et al.*, 2007; Miller *et al.*, 2012). Further, such studies have shown that basidiomycetes that expand in soil influence the plant community by suppressing later colonizers

or by modifying environmental niches for some species or even modifying competitive relationships (Shantz & Piemeisel, 1917; Elliott, 1926; Cosby, 1960; WANG *et al.*, 2005; Xu *et al.*, 2011).

Increase in soil hydrophobicity from hyphal expansion (Gramss *et al.*, 2005), immobilization of nutrients (Fisher, 1977), pathogenic behavior (Terashima *et al.*, 2004; Fidanza *et al.*, 2007), release of myogenetic phytotoxic compounds like cyanide (Blenis *et al.*, 2004; Caspar & Spiteller, 2015), and soil microbiota disequilibrium (Bonanomi et al. 2012), have all been proposed as triggering mechanisms of the detrimental behavior of FFR with respect to plant communities in grasslands. In parallel, causative mechanisms of the structuration of the plant community after FFR passage follow the same hypothetical approaches, such as disruption of existing pathogenic guilds, release of nutrient from dead microbial biomass, formation of vacant niches, or biosynthesis of plant hormones known as "fairy chemicals" (Choi, J-H *et al.*, 2010; Caspar & Spiteller, 2015; Suzuki *et al.*, 2016).

While the impact of FFRs on soil chemistry is well known (Gramss *et al.*, 2005; Fidanza *et al.*, 2007), little is known about the modification exerted on the fungal and bacterial microbiome. Moreover, the extent to which the FFR legacy persists during fungal progression is still obscure. Several studies have described the changes of fungi and bacteria in soil experiencing FFR growth (Ohara & Hamada, 1967; Kataoka *et al.*, 2012; Caesar *et al.*, 2013). Unfortunately, early studies were limited by culture-based methods (Nesme *et al.*, 2016). Currently, molecular techniques boost the inferential power of works aimed at explaining microbe-mediated plant-soil interactions (Leff *et al.*, 2018).

Taken together, the available evidence indicates that FFR fungi likely act as keystone species in shaping the bacterial, fungal and plant community. The impact of FFR expansion on soil microbiota has been assessed in temperate grassland (Yang *et al.*, 2018b), as well as for symbiotic FFRs (ectomycorrhizal), like *Tricholoma matsutake* in *Pinus densiflora* forests (Kim *et al.*, 2014; Oh *et al.*, 2016; Oh *et al.*, 2018). However, the majority of previous studies have separately addressed either the effect of FFR fungi on the microbial community (Ohara & Hamada, 1967; Kataoka *et al.*, 2012; Kim *et al.*, 2013), soil chemistry (Fisher, 1977; Gramss *et al.*, 2005) or the plant community (Edwards, 1984; Bonanomi *et al.*, 2012), while attempts to simultaneously target soil microbiota and the plant community are still lacking. Aiming to overcome such limitations, we combined plant community analysis with next generation sequencing to explore the role of FFR in structuring a Mediterranean grassland ecosystem. The main objectives of our study were:

(iv) to assess the differences of soil microbial communities before and after the passage of FFR fungi;

(v) to describe the plant community response to FFR activity;

(vi) to define the whole impact of FFR fungi on the above- and below-ground ecosystem structure.

4.2.2. Material and methods

Study site

The study was carried out in the Puro-Rogedano-Valleremita mountains in species-rich calcareous grassland in central Italy (43° 17′26″ N, 12° 51′29″ E) at 869 m a.s.l. In the site, the presence of FFRs is clearly

recognizable from the changing vegetation (Figure 4.2.1). The same study area was previously used for other research into FFRs (Bonanomi *et al.*, 2012) and plant species richness (Bonanomi *et al.*, 2009). The study site is subject to periodic mowing for fodder production, and grazing is restricted in the fall to a few wild populations of ungulates. As a species-rich hotspot the habitat is subject to biodiversity conservation policy (EU Directive 6210 'semi-natural dry grassland and scrubland facies on calcareous substrates (Festuco-Brometalia - *important orchid sites)).

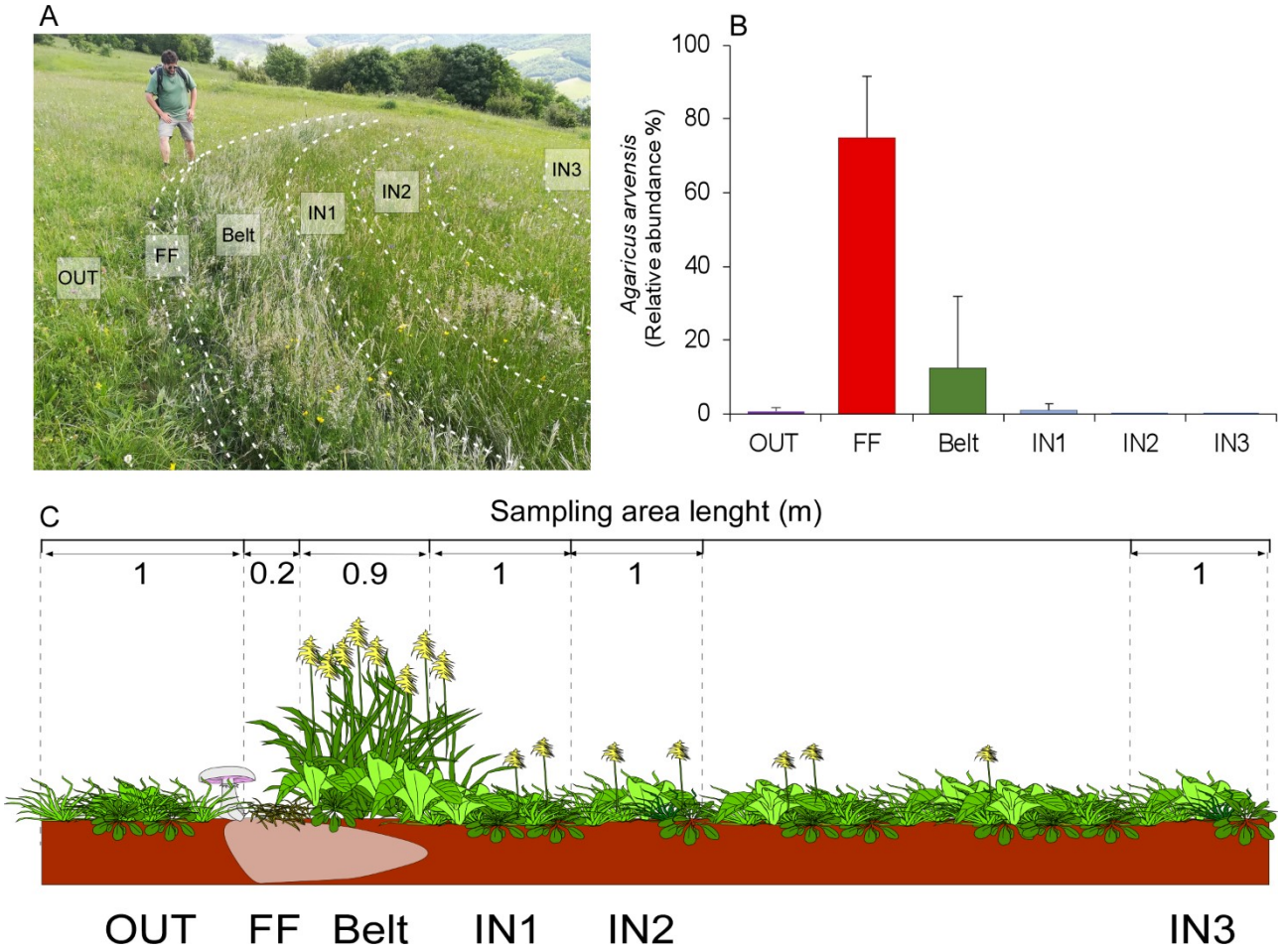

Figure 4.2.1 A) sampling zones across FFR of *A. arvensis* in Rogedano study site. B) Relative abundance of *Agaricus arvensis* in different sampling zones in the present study. C) Trasversal section of above and belowground aspect of grassland in presence of *A. arvensis* FFR. Diagram represent real length of zones of changing vegetation across the FFR.

The underlying soil is shallow and sandy (sand 75%, silt 12%, clay 13%), with an average depth profile of ~20 cm, and rich in organic matter (9.4%) and total N (8.7 g·kg-^{1}). The soil is characterized by pH 5.8, P_2O_5 9.4 mg·kg^{-1}, K_2O 118 mg kg^{-1}, Ca 2.9 mg kg^{-1}, C/N 10.7 and CEC (total cation exchange capacity) of 28.9 meq 100 g^{-1}. The soil has a low pH because it is a fersialitic paleosol that has been decarbonated. The mean annual rainfall is 945 mm, with a moderate dry season during the summer months that can lead to dry conditions in some years. The mean monthly temperatures are between 21.9 °C (July) and 3.8 °C (January) (means over 46 years of observations; Fabriano meteorological station, 357 m a.s.l., 7 km from the study site).

Vegetation analysis

Four regular shaped fairy rings were randomly selected and thereafter used for vegetation analyses. The ring-producing fungus was identified on the basis of sporophores morphological characteristics as *Agaricus arvensis*. FFRs in the area have been monitored for annual centrifugal advancement since 2008. The average fungal extension is ~60 cm yr^{-1}.

Here, in each ring four transects each 520 m long were used. Each transect consisted of contiguous 100 x 100 cm plots with the exception of FF zones which, given their small size, were 50 x 20 cm. A total of four transects composed of 24 plots were established. Six different zones were identified across transects, proceeding from the outer to the inner areas of rings. These are referred to as (Figure 4.2.1): Out = external zone not affected by the fungus; FF = fungal front identified by scorched vegetation and abundant, white fungal mycelium in the underlying soil; Belt = zone with lush, flourishing vegetation; IN1 = at a distance of one meter from the Belt zone within the FFR; IN2= at a distance of two meters from the Belt zone within the FFR; and IN3 m at a distance of five meters from the Belt zone within the FFR. The cover of each plant species along the transects, continuously monitored since 2008, was visually estimated during the growing season. Plant species determination followed the bibliographic repository of Italian flora. Plant cover was recorded with the Braun-Blanquet abundance–dominance scale transformed into percentages as follows: 5 = 85%; 4 = 60%; 3 = 35%; 2 = 15%; 1 = 5%; +=1%.

Soil sampling and DNA extraction

Six rings with similar characteristics and regular shapes were randomly selected and used for metagenomic analysis. Sampling points were identified with the same approach used for vegetation analysis. Sequentially, from outside to inside the FFR samples were collected and labeled as Out, FF, Belt, IN1, IN2 and IN3. In May 2017, soil samples were collected by means of a soil corer at a depth of 10 cm and put in sterile plastic bags. Between each sampling, the soil corer was thoroughly cleaned and sterilized to avoid sample contamination from exogenous microbial communities. Thirty-tree soil samples were collected. After collection, samples were rapidly frozen and stored at -80°C until the analyses. Total microbial DNA extraction from soil samples was carried out by using the DNeasy Power Soil kit (Qiagen) according to the manufacturer's instructions. Bacterial and fungal diversity was assessed by high-throughput sequencing of the amplified V3-V4 regions of the 16S rRNA gene (~460 bp) and ITS1-2 (~300 bp), respectively. Library preparation was carried out as previously reported (Bokulich & Mills, 2013; De Filippis *et al.*, 2017). PCR products were purified with the Agencourt AMPure beads (Beckman Coulter, Milan, IT) and quantified using an AF2200 Plate Reader (Eppendorf, Milan, IT). Equimolar pools were obtained prior to further processing and sequenced on an Illumina MiSeq platform, leading to 2x250bp, paired-end reads.

Sequence data analysis

Raw reads were filtered and analyzed by using QIIME 1.9.0 software (Caporaso *et al.*, 2010). Reads shorter than 300 bp, with more than one primer mismatch and with an average quality score lower than 25 were discarded. OTUs were picked through a *de novo* approach and the uclust method, and taxonomic

assignment was obtained by using the RDP classifier and the Greengenes (McDonald *et al.*, 2012) or the Silva SSU/LSU rRNA gene database release 119 (Quast *et al.*, 2012). Chloroplast and Streptophyta contamination, as well as singleton OTUs, were removed and the relative abundance of other taxa was recalculated. In order to avoid biases due to the different sequencing depth, OTU tables were rarefied to the lowest number of sequences per sample. The 16S and 18S rRNA gene sequences are available at the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI), under accession number PRJNA573680.

Data analysis

Statistical analyses and plotting were carried out using Primer 7 software (PRIMER-E Ltd, Plymouth; UK). Alpha diversity metrics were calculated analyzing community datasets in Primer 7. Heatplots were generated in Primer 7 software; samples were reordered according to the results of Bray-Curtis similarity clustering and variables according to the index of association similarity. The best 50 species with a major contribution in relative abundance to plant, fungi and bacterial communities are shown in heatplots.

A resemblance matrix calculated on Bray-Curtis dissimilarity was used to perform non-metric multidimensional scaling (nMDS) to assess variation in species composition across sampling points for plant, bacterial and fungal communities. In association with nMDS, significant changes in the three communities analyzed and alpha diversity were estimated through Permanova (999 permutations), using as fixed factor the sampling zones across FFRs.

We further analyzed functional group variation across the FFR in each community in question. We used different ordination methods: 1) For the plant community, the dataset was reordered according to family taxonomic level and maximum life span, namely perennials, biennials and annuals; 2. For the fungal community, a rarefied OTU table was submitted to FUNGuilds tools to evaluate trophic strategies of each fungal taxon available in the Unite Database (Nguyen et al., 2016); 3. For the bacterial community, given the high frequency of gene loss, duplication, or gene transfer which decrease the power of taxonomic and guild ordinations we performed PICRUSt analysis to predict functional bacterial process variation during FFR development (Langille et al., 2013). For each community, dataset produced was percentage transformed according to total values of each variable in the transects. In order to assess association between plant, fungal and bacterial taxa of the three reordered or predicted datasets and sampling zone we performed principal component analysis (PCA).

4.2.3. Results

Plant community composition

A heatplot ordered by Bray-Curtis dissimilarity among sampling zones showed differentiation in the plant community along FFR transects through significant changes in the relative abundance of plant species (*p-value* 0.001) (Figure 4.2.2A). In detail, the plant community outside the FFR (Out zone) is characterized by a dominant population of *Bromopsis erecta* (53.75%) and *Briza media* (12.50%) and less present *Festuca circummediterranea and Festuca gr.* (10.0 and 5.0%, respectively). In FF zones, corresponding to the higher level of abundance of *Agaricus arvensis* (Figure 4.2.1B), the plant community is at a lower level of similarity with respect to the other sampling zones in the transects (<25.0%) with a similarity of 21.45% with respect to

previous zone (Out) (*p-value* 0.002). Community differentiation is given by a generalized decline of the whole plant community present in the sampling site, with the sole exception of the increase in *Knautia calycinia* comprising 36.25% of the total plant community and *Betonica officinalis* increasing from 2.75% to 4 % of contribution in the plant community structure in FF zones. In the Belt zone, community composition showed a high level of differentiation with respect to the previous zone with low levels of similarity (17.53 % *p-value* 0.003). The plant community in the Belt zone differed in the presence of *Cynosurus echinatus* (35.0%)*, Bromopsis erecta* (15.0%)*, Cerastium glutinosum* (17.50%)*, Anthoxanthum odoratum* (15.0%) and *Festuca gr* (5.0%). Within the ring the IN1 zones showed similarity values of 36.63% with Belt zones (*p-value* 0.01), indicating a less abrupt change in the plant community which is dominated by *Lolium perenne* (30.0%) and *Trifolium incarnatum* (18.25%), nd with the persistence of *C. echinatus* (17.50%) and *C. glutinosum* (15.0%). A high level of similarity is instead observed between zones IN1 and IN2 (54.05%, *p-value* 0.281) with non-significant changes in the plant community structure experiencing the recurrence of *Festuca gr* at notable levels of abundance (10.0%). Lower levels of similarity are also observed when shifting from the IN2 to IN3 plant community (21.64% *p-value* 0.004) whereas a high level of similarity is observed between IN3 and Out (61.28%, *p-value* 0.699), indicating that the plant community structure is restored inside the rings with a community consisting of *B. erecta* (48.75%), *C. ambigua* (10.0%), *F. circummediterranea* (7.50 %), *B. media* (8.0%), and *Trifolium ochroleucon* (6.50%).

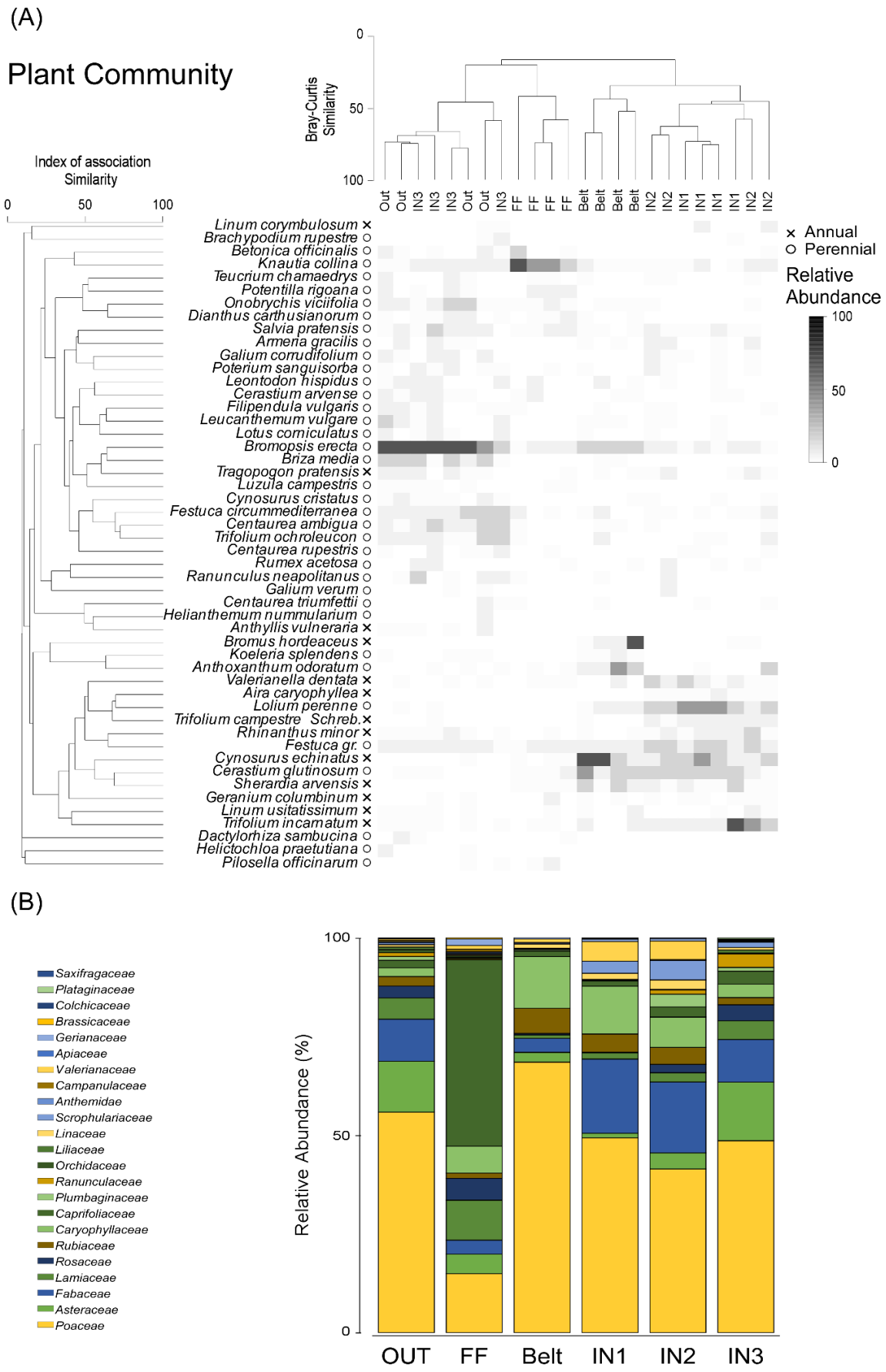

Figure 4.2.2 A) Heatplot of relative abundance of different plant species in plant community across FFR. Hierarchical clustering of samples is based on Bray-Curtis , while plant species are oredered according association index. B) Bar plot of relative abundance of different plant families across FFR of A. arvensis.

Analyzing the different communities at higher taxonomic level, we observed a clear-cut turnover in plant community structure (Figure 4.2.2B). Prior to the passage of FFR (Out), the community is mainly characterized by Poaceae (55.90%), followed by *Asteraceae* (12.90%), *Fabaceae* (10.60%), *Lamiaceae* (5.40%) and *Rosaceae* (3.0%). At the fungal passage (FF zone), the community shifted towards a structure where *Caprifoliaceae* become heavily dominant (47.2%), with a subsequent secondary site-associated family structured by *Poaceae* (15.0%), *Lamiaceae*, *Caryophyllaceae* and *Rosaceae* (10.8, 6.80 and 5.50%, respectively). In the following zones, a generalized legacy effect of FFR is appreciable with an imbalance of the original community structure for each zone. The high level of contribution of *Poaceae* (68,50%), *Caryophyllaceae* (13.10%), *Rubiaceae* (6.40%), and *Fabaceae* (3.50%) is attested in the Belt zone. In IN3, the community mainly cons by *Poaceae* (49.30%) *Fabaceae* (18.0%), *Caryophyllaceae* (12.10%), *Valerianaceae* (5.0%) and *Rubiaceae* (4.60%). In IN2 zone, community structure is almost similar to those of the previous zone (IN1) being composed by Poaceae (41.50%), *Fabaceae* (17.90%), *Caryophyllaceae* (7.70%) and *Scrophulariaceae* (4.90%). In the IN3 zone, similar hierarchical contributions of families present in the Out zone are appreciable, with *Poaceae*, *Asteraceae* and *Fabaceae* at 48.60, 14.80 and 10.80% of the total, respectively.

Table 4.2.1 Result of Permanova significance test across FFR sampling zones as fixed factors (N° of permutation 999). Reported significance of pairwise changes between contiguous sampling zones across the FFR of A. arvensis in plants community of Rogedano grassland ecosystems. Test of significance is based on alpha diversity metrics. Significance level is fixed for p-value below 0.05.

	S		H'(Log_2)		J'	
	t	*p-value*	*t*	*p-value*	*t*	*p-value*
Out *vs* FF	5.8427	***0.001***	2.0819	*0.08*	0.70783	*0.522*
FF *vs* Belt	1.7823	*0.12*	0.52301	*0.624*	0.14457	*0.864*
Belt *vs* IN1	0.73855	*0.473*	0.9641	*0.383*	1.2214	*0.291*
IN1 *vs* IN2	4.371	***0.002***	5.0874	***0.003***	4.6387	***0.005***
IN2 *vs* IN3	4.7001	***0.003***	0.88469	*0.404*	2.3825	*0.072*
IN3 *vs* Out	0.15133	*0.891*	0.51753	*0.616*	0.45849	*0.646*

Fungal community composition

A heatplot of relative abundances in different sampling zones across FFR showed that fungal abundances are concentrated in few taxa (Figure 4.2.3A), with some cases in which FFR zones do not show an appreciable predictive ability of the fungal community, although significant changes in fungal community composition are observed (*p-value* 0.001). The Out zone has a community consisting of unidentified fungi (24.0%), *Hygrocybe genus* (21.0%), *Ascomycota other* (15.0%), *unidentified Clavariaceae* (5.0%) and *Morteriella* (3.0%). From outside the grassland the development of *Agaricus* imposes significant changes in the fungal community (*p-values* 0.001) where the FR-forming fungus reaches considerable abundance levels (85.38%) (Figure 4.2.3B) and is associated with the presence of *Trichoderma* genera (6.57%). The overall changes in

the community is also assessed by the low level of similarity between the communities analyzed (9.83%). In the Belt zone significant changes in community are observed with respect to the FF zone (*p-values* 0.007) although higher values of similarity are recorded compared to the previous transition between sampling zones (24.52%). In the Belt zone the community consists in a large portion of unidentified fungi (14.0%), *Ascomycota other* (13.0%), a residual presence of *Agaricus* (12.0%), and in lower abundance by unidentified *Mixotrichaceae* (6.0%), *Mycena* (6.0%), *Clitocybe* (5.0%) and *Chetomium* (3.0%). Higher similarity (43.55%) and lower significance (*p-value* 0.047) are observed between the Belt and IN1 zones. The fungal community in Zone IN3 remains dominated by unidentified fungi (20.0%), *Ascomycota other* (12.0%) and the newly appearing Agaricales order (14.0%). Other taxa with a lower contribution are *Morteriella* (6.0%) and unidentified *Myxotrichaceae* (3.0%). In the inner zones of the FFR no significant changes are observed in the transitions through transect zones. In parallel, higher levels of similarity with respect to the more external zone of the rings is recorded between IN1 and IN2 (48.90% *p-value* 0.566), and IN2 and IN3 (48.62% *p-value* 0.269). Finally, the innermost community IN3 showed no evident changes with respect to the external grassland (Out) (*p-value* 0.780) with similarity at 47.74%.

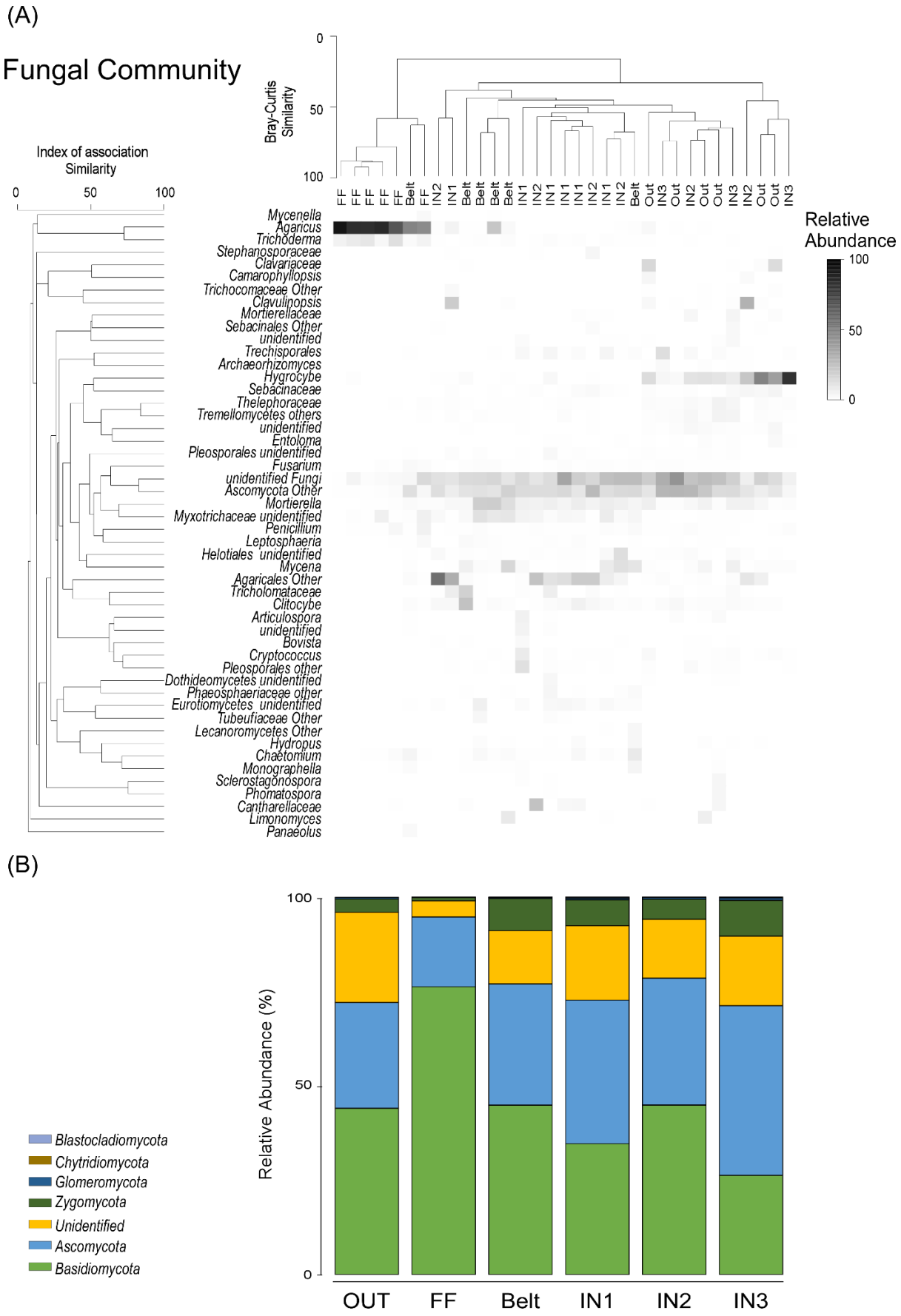

Figure 4.2.3 A) Heatplot of relative abundance of different fungal taxa in fungal community across FFR. Hierarchical clustering of samples is based on Bray-Curtis Similarity, while fungal taxa are oredered according association index. B) Bar plot of relative abundance of different fungal phylum across FFR of *A. arvensis*.

When analyzed at phylum level the differentiation among community structures showed comparable patterns with those highlighted in the heatplot, with few variations appreciable only in the case of the FF zone (Figure 4.2.3B). In detail, the Out zone was dominated by the Basidiomycota phylum (44.1%), Ascomycota (28.0%) and a large fraction of unidentified fungi (23.9%), with smaller contributions from Zygomycota (3.5%) and Glomeromycota (0.5%). With FFR development (FF zone), a sharp increase in Basidiomycota is recorded at the expense of all other phyla. Within the FFR (Belt, IN1, IN2) the organization of phyla is restored to similar level of the Out zone, with exceptions made for Zygomycota and Ascomycota which, on average, had a higher level of contribution within the FFR. The increase in Zygomycota and Ascomycota were associated with the decrease in Basidiomycota fungus which, in the last sampling zones, has lower levels of contribution with respect to the Out zone (26.2%).

Table 4.2.2 Result of Permanova significance test across FFR sampling zones as fixed factors (N° of permutation 999). Reported significance of pairwise changes between contiguous sampling zones across the FFR of *A. arvensis* in fungal community of Rogedano grassland ecosystems. Test of significance is based on alpha diversity metrics. Significance level is fixed for *p-value* below *0.05*.

	N°of OTU		H'(Log_2)		J'	
	t	*p-value*	*t*	*p-value*	*t*	*p-value*
Out *vs* FF	2.7184	***0,02***	4.2355	***0,005***	4.2812	***0,002***
FF *vs* Belt	5.1174	***0,001***	5.7176	***0,001***	5.4573	***0,001***
Belt *vs* IN1	0.64858	*0,546*	0.86171	*0,399*	0.8278	*0,407*
IN1 *vs* IN2	1.8839	*0,092*	2.1739	*0,063*	2.134	*0,072*
IN2 *vs* IN3	0.13993	*0,899*	0.52885	*0,608*	0.60266	*0,567*
IN3 *vs* Out	0.7622	*0,482*	0.71338	*0,497*	0.68438	*0,52*

Bacterial community composition

The heatplot of the bacterial community is characterized by a structure in which abundances are widely distributed among bacterial genera (Figure 4.2.4A), showing significant changes in community features between sampling zones of the FFR (*p-values* 0.001).

Starting from outside toward inside the FFR, the community has a higher portion of *DA101* (14.88%) followed by *Koribacteriaceae* (3.29%), *Candidatus Solibacter* (3.20%), *WD2101* (3,11%), *Rhodospirillaceae* (2.84%), *Rhodoplanes* (2.60%) and *Chitinophagaceae* (2.27%) in the Out zone. Correspondently with the developments of *Agaricus*, in the FF zone community developed as structure that is dominated by the presence of the *Bulkolderia* genus (26.57%), followed by *Sphingobacteriaceae* (7.96%), *Sphingomonas* (6.41%), *Actinoallomuros* (4.43%), *Streptomyces* (3.79%), *DA101* (2.77%), *Luteibacter* (2.68%), and *Bradyrhizobium* (2.64%). Modification in the organization of the community resulted in a lower level of Bray-Curtis similarity (26.76%) between the external grassland (OUT) and the zone with higher influence of the basidiomycete (FF) (*p-values* 0.001). Further significant changes are recorded when passing from the FF zone to the Belt zone (*p-*

values 0.001), with similarity levels higher with respect to the transition from Out to FF. Higher levels of similarity between FF and Belt (44.43%) are due to the analogous composition of the community at taxa level but with different values of abundance. *Sphingobacteriaceae, Chitinophagaceae* and *Actinoallomuros* are the most abundant taxa (4.50, 4.48 and 4.26%, respectively) followed by less abundant *Rhodospirillaceae* (2.71%), *Bulkolderia* (2.66%) and newly appearing *Herminiimonas* (2.70%), *Sphaerisporangium* (2.66%) and *Flavobacterium* (2.15%).

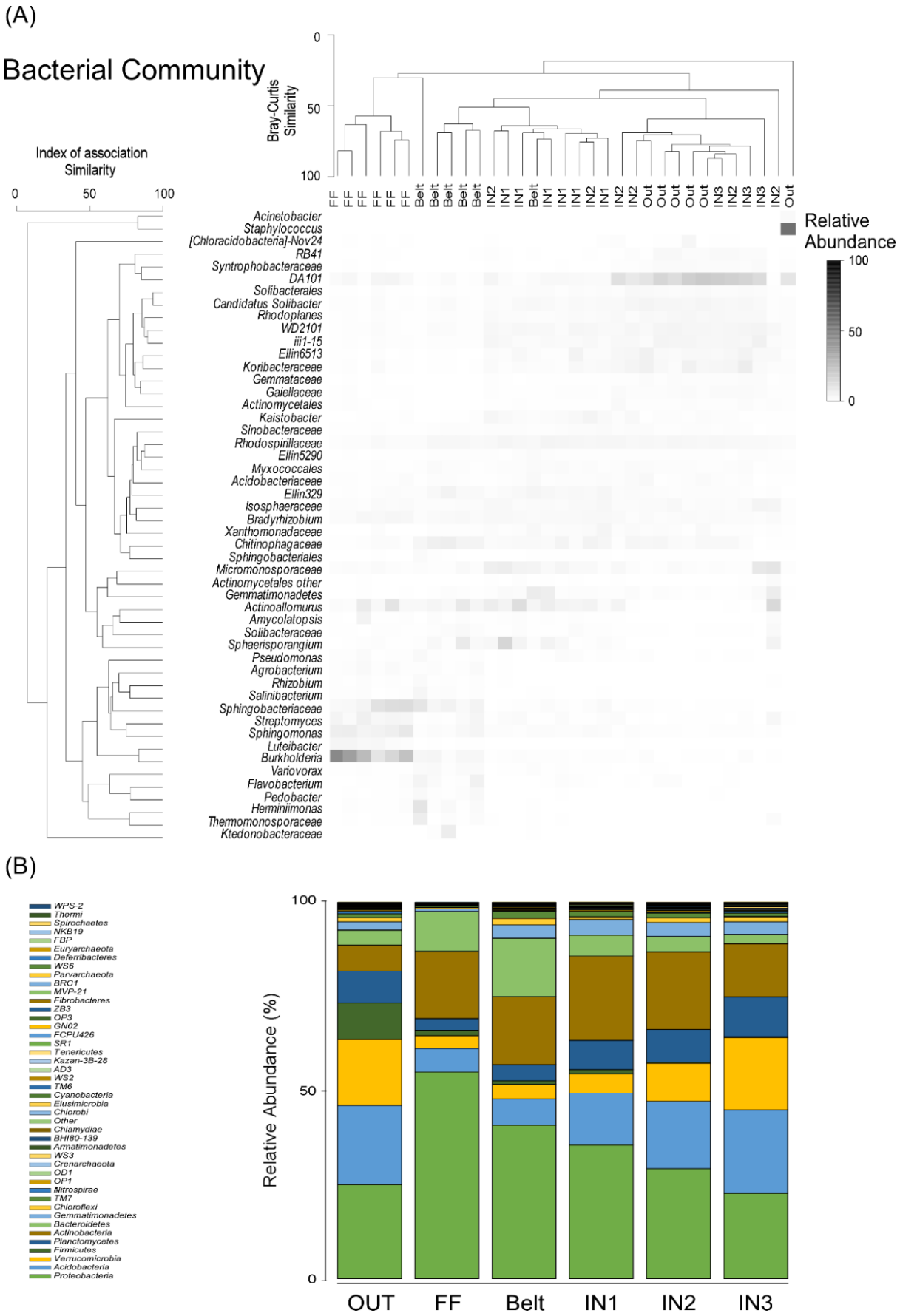

Figure 4.2.4 A) Heatplot of relative abundance of different bacterial taxa in bacterial community across FFR. Hierarchical clustering of samples is based on Bray-Curtis Similarity, while bacterial taxa are oredered according association index. B) Bar plot of relative abundance of different bacterial phylum across FFR of A. arvensis.

The next IN1 zone has an increased level of similarity with respect to the previous zone (56.91%) and less significant changes in the community (*p-values* 0.01). The main taxa contributing to community organization are *Actinoallomurus* (5.72%), *Sphaerisporangium* (4.96%), *Micromonosporaceae* (3.50%), WD2101(2.62%) and *Chitinophagaceae* (2.58%). Increasing similarity trends are also observed between sites IN1 and IN2 (65.50%, *p-values* 0.213) where the recurrence of *DA101* is noted as the main contributing taxon (7.57%) and the persistence of the presence of *Actinoallomurus* (5.18%) followed by *Micromonosporaceae* (3.95%), *WD212* (3.07%) and *Rhodospirillaceae* (2.84%). The bacterial community is maintained at the same level of organization of the IN2 zone in the more internal area of the FR (IN3) (54. 51% *p-values* 0.208) and becomes highly similar to the external grassland with 72,58% of similarity and no significant changes in community (*p-value* 0.533). The IN3 bacterial community comprises *DA101* as the main taxon (16.65%), followed by *Koribacteriaceae* (4.17%), *WD2101* (3.82%), *Rhodoplanes* (2.89%), *Candidatus Solibacter* (2.84%) and *Rhodospirillaceae* (2.56%)

At phylum level a considerable change is recorded in the soil microbial community after the passage of the FR forming fungus (Figure 4.2.4B): grassland soils outside the FFR (Out) host mainly *Proteobacteria* (24.9%), followed by *Acidobacteria* (21.1%), *Verrucomicrobia* (17.5%), *Firmicutes* (9.7%), *Planctomycetes* (8.4%), *Actinobacteria* (6.9%) and *Bacterioidetes* (4.0%). In the FF zone, *Proteobacteria* (54.9%), *Actinobacteria* (17.9%) and *Bacterioidetes* (10.5%) increase their abundance from the previous stage of the community, while *Acidobacteria* (6.2%), *Verrucomicrobia* (3.4%) and *Plantomyces* (3.1%) experience a generalized decline. In the next zones (Belt, IN1, IN2 and IN3), the main phyla contributing to community composition are arranged with the same structure described for FF zone until a composition similar to that of the external grassland is gradually reached. This effect, within the FFR, occurs at the expense of *Proteobacteria*, which gradually decline from a 40.8% contribution in the Belt to 22.7% in the last zone IN3, and *Bacterioidetes*, which decrease from 15.5% in the Belt to 2.5% in IN3.

Table 4.2.3 Result of Permanova significance test across FFR sampling zones as fixed factors (N° of permutation 999). Reported significance of pairwise changes between contiguous sampling zones across the FFR of *A. arvensis* in bacterial community of Rogedano grassland ecosystems. Test of significance is based on alpha diversity metrics. Significance level is fixed for *p-value* below *0.05*.

	S		H'(Log_2)		J'	
	t	*p-value*	*t*	*p-value*	*t*	*p-value*
Out *vs* FF	5.8427	***0.001***	2.0819	*0.08*	0.70783	*0.522*
FF *vs* Belt	1.7823	*0.12*	0.52301	*0.624*	0.14457	*0.864*
Belt *vs* IN1	0.73855	*0.473*	0.9641	*0.383*	1.2214	*0.291*
IN1 *vs* IN2	4.371	***0.002***	5.0874	***0.003***	4.6387	***0.005***
IN2 *vs* IN3	4.7001	***0.003***	0.88469	*0.404*	2.3825	*0.072*
IN3 *vs* Out	0.15133	*0.891*	0.51753	*0.616*	0.45849	*0.646*

3.1 Plant and microbial Alpha diversity changes within an FFR

Across the sampling zones, a generalized decrease in all the diversity metrics adopted in our analysis was observed from outside to inside the FFR, although in some cases such variation did not exceed significance thresholds. Overall, the highest degree of variation occurred in the FF zones where the active mycelium of Agaricus has higher relative abundance. In the site just behind the area of influence of Agaricus, a gradual restoration of the diversity metrics was appreciable, with complete reestablishment of the grassland communities in the more internal zones of the FFR (Figure 4.2.5).

In detail, generalized significant variations were found across FFR zones in plant species richness (S, *p-value* 0.001) and Shannon index value (H', *p-value* 0.003). Changes in species evenness (J') had no overall significance, although the shift from IN1 and IN2 zones showed a significant transition from a zone with less equitability to one with higher values (*p-values* 0.005).

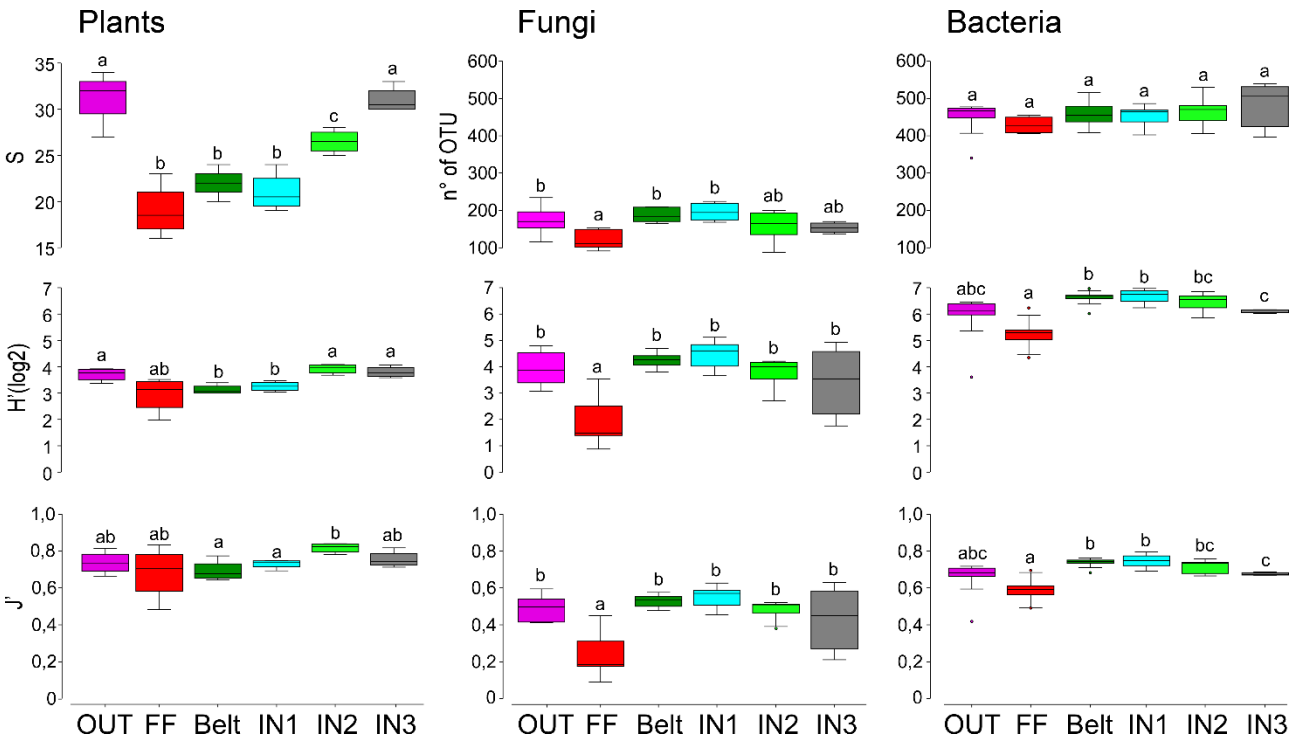

Figure 4.2.5 Box plots showing distribution of diversity indexes for each sampling zone across FFR of A. arvensis. S: Number of species, N° of OTU: Number of Operational Taxonomic Units, H': Shannon Index, J': Pilou's Evenness for plant, fungal and bacterial community

Table 4.2.4 Result of Permanova significance test across FFR sampling zones as fixed factors (N° of permutation 999). Here reported general significant variation across the FFR of different Alpha diversity metrics in plants, Fungal and Bacterial communities of Rogedano grassland ecosystems. Significance level is fixed for p-value below 0.05.

	S/N°of OTU		H'(Log_2)		J'	
	Pseudo-F	***p-value***	***Pseudo-F***	***p-value***	***Pseudo-F***	***p-value***
Plants	23,211	***0.001***	5.5937	***0.003***	1.7942	*0,171*
Fungi	4.4829	***0.007***	8.8985	***0.001***	9.1041	***0.001***
Bacteria	0.83748	*0.543*	5.2633	***0.003***	5.5896	***0.001***

The changes in the fungal community in species richness indexes resulted in significant variations for each metric (*p-value* at 0.004, 0.001 and 0.001 for OUT number, H' and J', respectively). All the metrics dramatically decreased from outside the ring following fungal development (FF), with subsequent recovery of fungal diversity in the inner zones.

Diversity indexes for bacterial community showed evident changes in species richness (H') and species equitability (J') (*p-value* 0.002 for both indexes) across FFR, while no significant variation was found for the number of OTU (*p-value* 0.510). In both the Shannon index and community evenness, the Out zone showed

high heterogeneity in species distribution which was restricted and decreased with FFR development (FF). Within the FFR, values of the metrics, quickly recovered reaching maximum values in Belt and IN1 zones and decreased with a particular narrow distribution in zones IN2 and IN3.

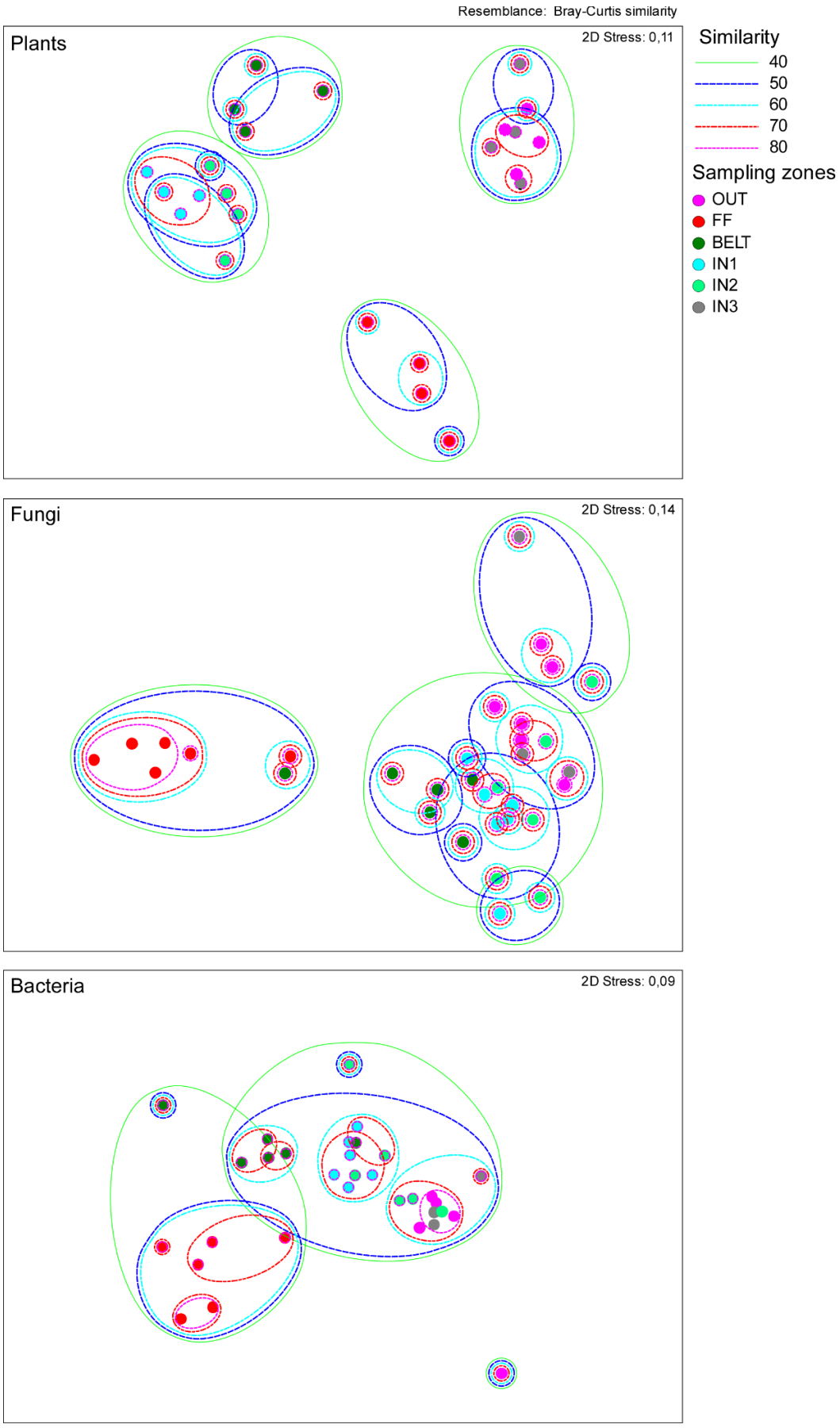

Figure 4.2.6: Non-metric Multidimensional Scaling based on Bray-Curtis similarity values showing changes in of Plant, Fungal and Bacterial communities in transect across FFR of A. arvensis.

Table 4.2.4 Result of Permanova significance test across FFR sampling zones as fixed factors (N° of permutation 999). Here reported general significant variation (Main) and significance of pairwise changes between contiguous sampling zones across the FFR of A. arvensis in plants, Fungal and Bacterial communities of Rogedano grassland ecosystems. Test of significance is based on Bray-Curtis similarity values. Significance level is fixed for p-value below 0.05.

	Plants		Fungi		Bacteria	
	Pseudo-F/t	*p-value*	*Pseudo-F/t*	*p-value*	*Pseudo-F/t*	*p-value*
Main	7.6085	**0.001**	6.4154	***0.001***	7.7807	***0.001***
Out *vs* FF	3.1393	***0.001***	5.1398	***0.001***	4.1434	***0.001***
FF *vs* Belt	2.8666	***0.001***	3.7403	***0.002***	2.9041	***0.001***
Belt *vs* IN1	2.1872	***0.012***	1.4856	*0.055*	2.086	***0.005***
IN1 *vs* IN2	1.151	*0.305*	0.88573	*0.589*	1.2099	*0.202*
IN2 *vs* IN3	2.7317	***0.004***	1.1403	*0.265*	1.2695	*0.188*
IN3 *vs* Out	0.69996	*0.699*	0.65947	*0.774*	0.8206	*0.544*

Plant and microbial functional aspects

Principal component analysis (PCA) of the plant community dataset according to lifespan showed a clear pattern associated to FFR development in grassland. Overall, PCA explains 57.9% of the total variance in the dataset (PCI: 37.6% and PCII: 20.3%) (Figure 4.2.7A). In the Out zone the plant community is strongly associated with perennial plants. The action of the fungus creates conditions for the establishment of a community that mainly consists of annuals. Following the action of the FFR, association between annual plants and the internal zones of the rings Belt, IN1 and IN2 are observed. In the inner zone of the ring (IN3), the perennial-dominated pattern is reestablished.

PICRUSt analysis of the dataset afforded an overview of how the bacterial community is structured to cope with different conditions occurring in different FFR zones (Figure 4.2.7B). PCA of the dataset obtained from dataset reordination explains an intrinsic variance in the data of 96.9%, with the highest portion of variability explained on the first component (92.3%), and a lower explanatory power for the second component (4.6%). Among the 32 variables employed in our data analysis, only the variable of xenobiotic biodegradation and metabolism showed a clear pattern of association with the FF, Belt and less strongly with IN2. By contrast, sampling zones IN1, Out and IN3 showed a generalized overlap of bacterial cellular processes.

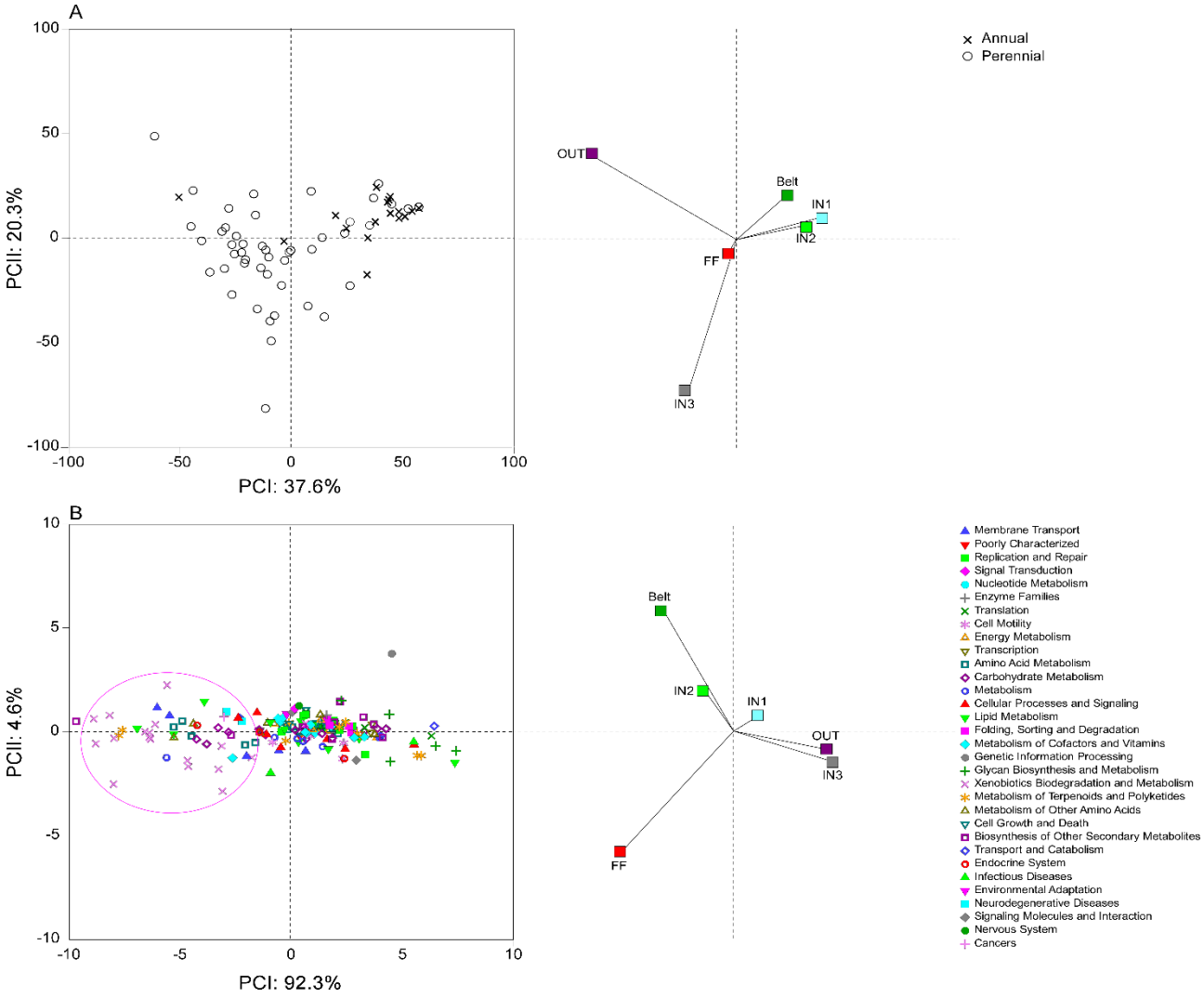

Figure 4.2.7 Principal component analysis (PCA) of plant community. Point shape indicate plant life span (A). PCA based on PICRUSt predicted KEGG pathways for bacterial community (B). Both datasets were variable standardized.

In parallel, the PCA of dataset on the fungal community following the assignment of fungal guilds explained 58.2% of the total variance (PCI:31.1 % and PCII: 27.1%). Among the six guilds associated with sampling zones a clear association was observed between arbuscular mycorrhizal taxa and the internal zones of the FFR. A strong association was observed in the Belt and IN1 zones, while lower correspondence was recorded within zones FF, IN2 and IN3 (Figure 4.2.8).

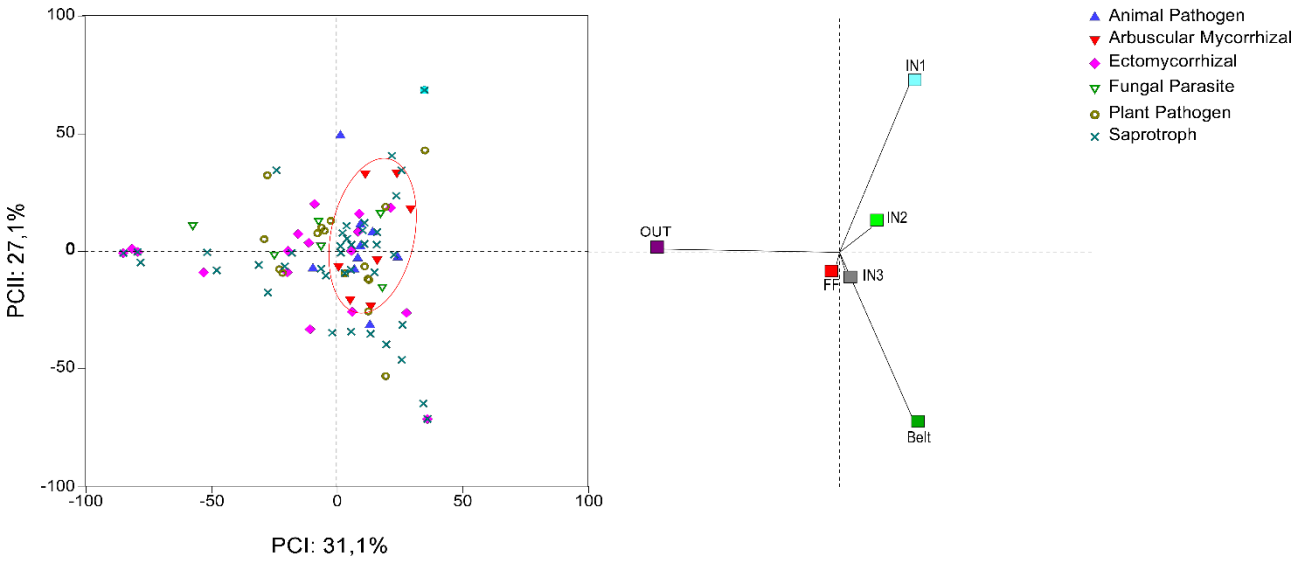

Figure 4.2.8 Principal component analysis (PCA) based on fungal community composition. Point shape indicates the classification of the taxon based on FUNGuild assignment. Dataset was variable standardized.

4.2.4. Discussion

Plant community response to FFRs

Our results showed a fungal-dependent shift in plant community structures operated by a wave-like spread of FFRs in grassland soil. We detected a general decrease in plant diversity metrics, attesting a detrimental effect of FFRs on most plant species rooted in the path of the fungus. Moreover, we detected a legacy of FFR action on grassland, causing an increase in plant species richness after its passage due to the complete plant turnover and subsequent community recovery.

Our finding is in accordance with previous works focusing on Type-1 FFR, especially with those recognizing the *Agaricus* genus as the driver of FFR phenomena (Shantz & Piemeisel, 1917; Edwards, 1984; Bonanomi et al., 2012). Moreover, we found a legacy effect on the plant community structure, indicating that, at an annual growing rate of the FFR of around 60 cm, the plant community is resilient, although several years are required to restore the structural composition of unaffected grassland: the development of the FFR has a detrimental effect on the abundance of perennial species and especially on dominant perennial Poaceae, as also described in American grasslands (Cosby, 1960). The mechanisms causing the death of most of the plants is still a matter for debate. Induction of soil hydrophobicity from accumulation of dense mats of mycelium (Gramss et al., 2005), direct pathogenic activity (Terashima et al., 2004), and release toxins like cyanide (Blenis et al., 2004; Caspar & Spiteller, 2015) are among the most cited hypotheses. In this regard, it is remarkable that only one plant, *K. collina*, was able to resist the passage of the FFR. Escape from competition of dead perennial grasses and some specific ability to tackle the passage of the FFR could explain the persistence of *K. collina* in the FF zone.

After the passage of the FFR, grassland composition shows a total turnover with a shift towards short-lived, annual species. This phenomenon was already described in Type-1 FFR of *M. oreades* and *Agaricus*

spp. (Shantz & Piemeisel, 1917; Cosby, 1960; Bonanomi et al., 2012). Structural fluctuation of the plant community and subsequent gradual restoration remind to the typical response to intense abiotic disturbance, where short-lived plants rapidly recolonize the empty space (Pärtel et al., 2005; De Luis et al., 2006). Taken together, our results indicate that the sudden disappearance of a stable grassland community causes a competitive release where faster-growing and short-lived species can colonize the gaps created by the passage of the fungus. The possibility that short-lived species take advantage of the soil chemo-physical modification operated by FFRs cannot be ruled out (Bonanomi et al. 2012). In the inner ring zones, however, progressive restoration of the original plant community is observed.

FFR affects the fungal microbiome

To date, this is the first work to report the effect of FFR development on the fungal community with next generation sequencing technologies in grassland, with previous studies reported only for ectomycorrhizal *T. matsutake* FFR (Kim et al., 2013). Here, the fungal community assemblage was destabilized by the passage of the FFR, with a more pronounced simplification than that observed for the bacterial microbiome. In correspondence of *A. arvensis* vegetative mycelium we found the presence of only one fungal taxon belonging to the *Trichoderma* genus. The occurrences of *Trichoderma* in proximity of the FFR was also described for *T. matsutake* (Park et al., 2014; Oh et al., 2018). Trichoderma are a well-known fungal species heavily employed as beneficial organisms in biological crop protection (Vinale et al., 2008). The *Trichoderma* genus has high chitinolytic ability, suggesting that *A. arvensis* in FFR soils is followed by a weak parasite. This work is the first to report the association between *Trichoderma* and a fairy ring forming fungus in grassland, implying that this mycoparasitic interaction is common both for FFR developed in woodland and for those developed in grassland ecosystems. In the Belt zone of the ring, the *Mortierella* genus and *Myxotrycaceae* fungal family became more abundant. The result is consistent with similar works observing FFR development and effects on the fungal community when considering the *Mortierella* genus. Indeed, if the former is known for its chitinolytic activity comparable to that of the *actinomycetes* (Johnson et al., 2019), the latter are more associable to cellulolytic activity (Tribe & Weber, 2002), suggesting a competitive release for this family instead of a myco-parasitic advantage.

As observed for bacteria and higher plants, the fungal microbiome showed a quick recovery after the FFR passage. The ordination of the datasets by fungal functional groups showed changes in relative abundance according to sampling zone which, however, are not predictive for specific fungal guilds. The sole exception is for arbuscular mycorrhizal guilds increasing in the inner zone of the rings. This association should take into account that the newly formed plant community with enhanced growth could act as sink for accumulation of AM fungal symbionts.

FFRs alter grassland prokaryotic communities

As in the plant community, FFRs trigger a complete compositional change in grassland prokaryote assemblages. Expansion of *A. arvensis* operates through a simplification of the number of prokaryotic taxa and

their evenness. The effect of FFR passage profoundly changes the bacterial community, even if the outcome is less marked when considering the legacy of the fungal passage. Rapid recovery of the bacterial microbiome is likely due to the high replicative potential of bacterial species that quickly recolonize the inner zones of the ring. This effect is consistent with metagenomic-based studies describing a dramatic decline of bacteria in proximity to vegetative mycelium of *T. matsutake* ectomycorrhizal FFR (Kim *et al.*, 2014; Yang *et al.*, 2018b).

The simplification of the bacterial microbiome caused by FFRs is, however, characterized by the proliferation of few taxonomic groups. *Burkholderia* is the bacterial genus that becomes most abundant in the FFR front, followed by *Streptomyces*, *Actinoallomurus* and the *Sphingobacteriaceae* family with the related *Sphingomonas* genus. Most of the works emphasizing the above change imposed by FFR development in the bacterial community describe such taxa as potential mycorrhizal helper bacteria as they are often associated with, and recovered from, the active growing zone of ectomycorrhizal FFR (Kim et al., 2014; Oh et al., 2016). In contrast with the above studies, we suggest a potential mycoparasitic role of such bacteria in light of specific culture-based works demonstrating no effect or antifungal activities towards some FFR species (Oh & Lim, 2018a). The assumption is the same as we already suggested for the fungal community and is further supported by the fact that some of these bacteria are commonly applied as fungal suppressors of plant pathogens in agroecosystems (Holmes et al., 1998). Additionally, the mycoparasitic relation among such bacterial genera and fungi was supported by studies on *Streptomyces* and *Burkholderia* (Trejo-Estrada et al., 1998; Govan, 2000). Subsequently, the species that depend on living mycelium of the FFR are progressively replaced in the inner part of the ring by chitinophagous species. Plausibly, this indicates that the degradation of senescent mycelium of *A. arvensis* is a process affecting the microbial community because of the large amount and persistence of senescent mycelium in soils, with a lasting legacy effect, as estimated from the distance from the BELT zones.

Our analysis also revealed a relation between zones experiencing early fungal development and biological patterns promoting degradation of xenobiotic-related molecules. In the BELT and FF zones we found an increase in xenobiotic biodegradative processes, while, in the undisturbed zone and in the inner zones of the rings, microbiome functions are mainly associated with constitutive molecular pathways (Figure 4.2.7). The results suggest that the up-regulation of detoxification processes could be associated with the release of toxic compounds from fungal mycelium which, in turn, should be the triggering factor of the perturbation operated by the FFR in the three communities subject to the study. Additionally, most of the bacterial taxa intervening in the fairy ring active zone have a potential effect in the degradation of xenobiotic compounds (McMullan et al., 2001). In particular, *Burkholderia* demonstrated ability to convert harmful mycotoxins into plant hormone precursors, suggesting that mycoparasitic bacteria developed evolutionary mechanisms to detoxify the elective trophic substrate (Choi et al., 2016). Indeed, further studies are needed to identify the nature of the chemicals released by FFRs into soil that trigger the observed changes of bacterial microbiome.

FFR and ecosystem diversity

Overall, we demonstrate that a community level FFR fosters species richness of higher plants as well as the soil microbiome in Mediterranean grassland. This is consistent with previous studies demonstrating that FFR increase higher plant gamma diversity in grassland ecosystems (Bonanomi et al., 2012). Moreover, from our results we conclude that, as occurring for the plant community, the effect is similarly present in the microbial community of the soil affected by the phenomenon. Indeed, we observed a limited number of plant and microbial species gaining advantage from the direct action of the FFR. Instead, a large number of plants and microbes take advantage of the competitive release and the environmental conditions generated by FFR passage. Hence, the process described is more similar to that reported regarding the effect of a niche enlargement from abiotic disturbances or to intermittent streams when considering that a discrete number of taxa depend on such phenomena to increase their abundance (Dodds et al., 2004; Kozlowski, 2012; Atwater et al., 2018).

From the mechanistic point of view, several works have collected evidence and formulated hypotheses regarding the functioning of the detrimental effect of Type-1 FFR on soil communities. To the best of our knowledge the various hypotheses may be listed as follows: change in the soil structure and texture as the conversion of soil into a hydro-repellent matrix (Shantz & Piemeisel, 1917; Gramss et al., 2005); a nutritive deficiencies hypothesis, where a fungus with its own activity exploits and immobilizes mineral resources available for the coexisting plant and microbial communities (Fisher, 1977; Edwards, 1988). Further hypotheses include the release of mycotoxins like cyanuric compounds or complexed chemical compounds with phytotoxic and antimicrobial ability (Elliott, 1926; Blenis et al., 2004), and, as described by some authors, a pathogenic behavior on the part of the Type-1 fairy ring fungi (Terashima et al., 2004). Although all of the above hypotheses may be considered plausible, what is still required is hierarchical ordination of such factors or a clear explanation of how FFR suppress higher plants, bacteria and fungi in soils. However, the hypotheses in question are not mutually exclusive, reinforcing the idea that a combination of such factors can act simultaneously and produce a Type-1 FFR effect in grassland communities.

Regarding the post-passage effect of FFR in grassland soils, various considerations can be made. After the suppressive effect in the zone of action of the FFR (FF), the process of community restoration could be slower or faster depending on whether the community consists of plants or microbial species. The decoupled time of restoration may lie in the concept that the plant community requires more time to recover with respect to the microbial community in relation to life span and replication capability. In the Belt zone plant growth is enhanced as also occurs in Type-2 FFR. This effect could be attributable to chemo-physical changes in the fungal secondary metabolite (Choi, J-H *et al.*, 2010; Choi *et al.*, 2016), or to the instauration of a beneficial microbial community such as that consisting of *Burkholderia*, *Streptomyces*, *Trichoderma*, *Morteriella*, and arbuscular mycorrhizal fungi. In this context, it would be interesting to investigate whether the spatial and temporal dynamics of FFR (i.e. its ring shape and spread through grassland) can be attributed to the release and accumulation of its own extracellular DNA (Mazzoleni *et al.*, 2015a) in the central part of the structure as suggested by the modeling work of (Vincenot *et al.*, 2017).

4.2.5. Conclusions

In the present work, we demonstrated that the FFR wave-like centrifugal spread in grassland induces drastic changes in the plant and soil microbiome community. The profound changes in grassland functioning, composition and diversity induced by a single species suggest that an FFR can be considered an ecosystem engineer species (Jones et al., 1997). The Type-1 FFR alteration shown acts at different levels, modulating the number of species and its evenness in the community. The phenomenon as described enhances species turnover, with taxa that uniquely appear or draw advantage from singular environmental conditions in each sampling zone across the FFR. This demonstrates that several plant and microbial species depend on the passage of the FFR, putting it at the same level as other ecological factors that allow species coexistence like fire, drought, grazing and phytotoxicity (van der Maarel & Sykes, 1993; Bonanomi *et al.*, 2005).

4.3. Cascade effect on soil microbiota by a Fairy ring fungus.

4.3.1. Introduction

Fungal fairy rings (FRs) are biological phenomenon caused by radial expansion of Basidiomycetes fungi in soil (Shantz & Piemeisel, 1917; Nelson, 2008). This kind of processes are ubiquitous in telluric ecosystems characterized by high inputs of plant death organic matter, occurring majorly in grassland, woodland and turfs (Gregory, 1982). The presence of a FRs in grassland is, in most of the cases, detected by the effect on vegetation or the circular disposition of basidiomycetes carpophores in circular or arc-like pattern (Karst *et al.*, 2016). With its radial expansion FRs modify soil physicochemical conditions and in turn produces changes in prokaryotic and eukaryotic community in affected soils (Ohara & Hamada, 1967; Kataoka *et al.*, 2012). With more detail, has been described that the decomposition of organic matter operated by the FR-fungus induce a cascade of effect as such as the increase in macronutrients like NH_4 (Fidanza *et al.*, 2007) and NO_3 (Hardwick & Heard, 1978), Accumulation of P, K and Mg (Fisher, 1977; Gramss *et al.*, 2005) but also induce change in soil physic properties as such as soil acidification by PH decrease (Gramss *et al.*, 2005; Bonanomi *et al.*, 2012), decrease of soil moisture by increase of hydrophobicity (Yang *et al.*, 2019) and degradation of soil humus (Mathur, 1970). In turn, the conditioning effect of the fungus on the chemical and physical feature of soil promote change in telluric biological community provoking community simplification that has been firmly described for soil Mycobiota (Kim *et al.*, 2013; Kim *et al.*, 2014), for plant community (Cosby, 1960; Hardwick & Heard, 1978; Edwards, 1984; Bonanomi *et al.*, 2012)) and with less concordant results for soil prokaryotic community (Ohara & Hamada, 1967; Kataoka *et al.*, 2012; Oh *et al.*, 2018; Xing *et al.*, 2018). Triggering factor promoting change in biological communities subject to FR passage are differently supported in literature. Some authors hypothesized that the effect of a FR is generated as a consequence of: 1) Increase in soil hydrophobicity by release of hydrophobins by FR mycelium in surrounding soil (Shantz & Piemeisel, 1917; Gramss *et al.*, 2005); 2) Direct pathogenic behaviour as such as that described for *Marasmius oreades* (Elliott, 1926; Blenis *et al.*, 2004); 3) Unbalance of plant beneficial microbial community in soil (Bonanomi *et al.*, 2012; Espeland *et al.*, 2013) ; 4) Toxic Nutrient Over enrichment (Edwards, 1988); 5) Release of toxic compounds as such as cyanides or metal-binding compounds (Blenis *et al.*, 2004; Nishino, 2017; Nishino *et al.*, 2017). The hypotheses accumulated on the mechanism of action of the FRs are focalized on the effect on plant community. For microbial community studies produced are majorly aimed to describe instead to check for qualitative correlations between variables. Indeed, the first studies on the FRs action on soil microbial communities was based on extensive culture-based methods recognizing different microbial species by morphological aspect of colony and excluding non-culturable species (Ohara & Hamada, 1967). More recently, the set-up of molecular biological techniques able to unravel the composition of microbial community in soil offer the opportunity to understand the cascade effect promoted by the passage of a FR in soil. In light of this, our work is aimed to furnish information regarding the relation between changes imposed by FR fungus on Physical and chemical characteristics of affected soil and the extent to which these variation affect telluric microbial communities.

4.3.2. Material and methods

Study Site

The study was carried out in botanical garden of IRENA, León Spain (42°34'59.84"N, 5°35'24.31"O) at 837 m a.s.l. In the site, FRs of different fungal species appears seasonally and are recognizable from the changing vegetation as Type I FRs, as in the case of *C. gambosa,* and carpophore appearance in Type III FRs for *Lepiota rachodes, Macrolepiota procera* and *Agaricus xanthodermus*. The study site is subject to periodic mowing and irrigation follow seasonal precipitation. Woody species present in botanical garden are mainly *Aesculus hippocastanum*, *Sorbus domestica, Cedrus atlantica, Quercus suber, Picea abies, and Populus nigra*.

The underlying soil is shallow and sandy (sand 59.3%, silt 32.6%, clay 8%), with an average depth profile of ~25 cm, and rich in organic matter (11.0%) and total N (0.59%). The soil is characterized by pH 6.74, P olsen 16.3 pmm, K 32,2 cmol*kg^{-1} and CEC (total cation exchange capacity) of 32.2 cmol*kg^{-1}. Further information regarding chemical composition of macro and micronutrients are available in Table 4.3.1

Vegetation analysis for determination of FR typology

Three regular shaped fairy rings were randomly selected and thereafter used for our survey. The FR-forming fungus was identified on the basis of sporophores morphological characteristics as *C. gambosa*. Here, in each ring three transects with a total length of 3 m were used. in Each transect consisted of contiguous 10 x 10 cm plots. A total of three transects composed of 9 plots were established. three different zones were identified across transects, proceeding from the outer to the inner areas of rings. These are referred to as: Out = external zone not affected by the fungus; ON = fungal front identified by scorched vegetation and abundant, white fungal mycelium in the underlying soil; IN = at a distance of one meter from the ON zone within the FR. From how described in other work the present FRs of *C. gambosa* are included in Type I because of the presence of barren soil with death vegetation in correspondence of the fungal mycelium and coupled to a subsequent belt of flushing vegetation. Unusually, the FRs studied in the present work showed two narrow belts of flushing vegetation in front of and behind of the ON zones.

Soil sampling, DNA extraction and chemical analysis

The same rings chosen for vegetation analysis were selected for application of metagenomic analysis. Soil samples were collected in correspondence of zones identified in the vegetation analysis and labeled as OUT, ON and IN. Sample collection was carried out in May 2019 and soil sampling was collected by means of a soil corer at a depth of 10 cm and placed in sterile plastic bags. Between each sampling, soil corer was thoroughly cleaned and sterilized to avoid sample contamination. A total of nine soil samples were collected. After collection, samples were rapidly frozen and stored at -80°C until the analyses. Total microbial DNA extraction from soil samples was carried out by using the DNeasy Power Soil kit (Qiagen) according to the manufacturer's instructions.

Bacterial and fungal diversity was assessed by high-throughput sequencing of the amplified V3-V4 regions of the 16S rRNA gene (~460 bp) and ITS1-2 (~300 bp), respectively. PCR was carried out with primers and conditions previously reported (Bokulich & Mills, 2013; Berni Canani *et al.*, 2017). PCR products were

purified with the Agencourt AMPure beads (Beckman Coulter, Milan, IT) and quantified using an AF2200 Plate Reader (Eppendorf, Milan, IT). Equimolar pools were obtained prior to further processing and sequenced on an Illumina MiSeq platform, leading to 2x250bp, paired-end reads.

Concomitantly with DNA extraction, a quota of soil for each sample was used for chemical analysis. Soil was sieved to remove coarse particles and delivered in a Leòn university facility for analysis of soils chemical compositions. Analysis of Nitrates and Ammonia was carried out directly after sampling in order to avoid environmental exposition of the sample and alteration of the analytical results. Hydrophobicity test was performed through the drop test according to (Doerr, 1998) that consist in the time needed for a drop of water to be adsorbed by the sampled soil.

Sequence data analysis

Raw reads were filtered and analyzed by using QIIME 1.9.0 software (Caporaso *et al.*, 2010). Reads shorter than 300 bp or 150 bp (for Bacteria and Fungi, respectively), with more than one primer mismatch and with an average quality score lower than 25 were discarded. OTUs were picked through a *de novo* approach and the uclust method, and taxonomic assignment was obtained by using the RDP classifier and the Greengenes (McDonald *et al.*, 2012) or the UNITE v.8 database (Nilsson *et al.*, 2018). Chloroplast and Streptophyta contamination, as well as singleton OTUs, were removed and the relative abundance of other taxa was recalculated. In order to avoid biases due to the different sequencing depth, OTU tables were rarefied to the lowest number of sequences per sample.

Data analysis

Soil physical and chemical data were tested for significance through Univariate Analysis of variance (ANOVA) with significance level fixed at *p-value*s below 0.05. In order to avoid heteroscedasticity within the dataset significance was assessed on logarithmic transformed data. For biological parameters of microbial communities in the different *C. gambosa* FR zones alpha diversity metrics were calculated analyzing community datasets in Primer 7 and significance variation between sampling zone by pairwise comparison of result from Permutation Analysis of Variance (Permanova with Unrestricted permutation of data, 999 permutations). Significance level fixed with *p-value*s below 0.05. Heatplots were generated in Primer 7 software. In heatplots, samples reordered according to the results of Bray-Curtis similarity clustering and variables according to the index of association similarity. The seventy species with a major contribution in relative abundance fungi and bacterial communities are showed. A resemblance matrix calculated on Bray-Curtis similarity distance was used to perform Canonical correspondence analysis (CCA). Multivariate approach was used in order to produce data reduction and facilitate assessment of relations between taxa composition within each sampling points and changing soil physicochemical properties. Multiple correlation was performed to plot trajectories of most important associations (threshold fixed at correlation values below -0.3 and above 0.3 of R^2). Most important correlation was subsequently tested in order to describe the specific interaction between soil physicochemical characteristics and seventy most abundant microbial taxa isolated in each community (69 for fungal community excluding *C. gambosa*). Correlation was performed through Spearman Rank methodology. Results of Spearman rank correlation was both visualized as Heatplots and as unidirectional network of

interaction in Cytoscape (Smoot *et al.*, 2010) and undergo to network analysis of interactions. Datasets was also tested for group similarity and dissimilarity by applying Simper function in Primer-7 for both Least observed taxonomic level and phylum level. Previous to multivariate data analysis, datasets were Hellinger transformed. Significant changes in beta diversity data was tested for significance through permutational analysis of variance (Permanova with Unrestricted permutation of raw data, 999 permutations), using as fixed factor the sampling zones across FRs. Bonferroni correction was applied to avoid biases of multiple comparison. Multivariate data Analysis and multivariate significance tests were carried out using Primer 7 software (Clarke & Gorley, 2015). Univariate tests of significance based on ANOVA were performed through Statistica 10 software. Qualitative differences of species turn-over and overlap of microbiota in *C. gambosa* FR zones is assessed through Venn diagrams (Heberle *et al.*, 2015).

4.3.3. Results

Modifications of soil chemistry and physical parameters operated by C. gambosa

Results of chemical analysis and significant changes are summarized in (Table 4.3.1). Overly, 12 out of 24 chemical-physical characteristics of soil significantly varied in the active zone of the FR compared to those of soil outside and inside of the last. NH_4 (F 123.5, $p < 0.001$), NO_3 (F 13.7, $p < 0.01$) and K (F 5.7, $p<0.05$) are the three out of five macronutrients showing significative variation. Among micronutrients, six out of eight parameter showed significative variation for Mn (F 67.1 $p < 0.001$), Zn (F 56.0 $p < 0.001$), B (F 53.0 $p < 0.001$), Fe (F 23.3, $p < 0.01$) Cu (F 21.9, $p < 0.01$) and Mg (F 13.0, $p<0.01$). For physical parameters of soil, significative variations are observed in soil Conductivity (F 28.6 $p < 0.001$), Hydrophobicity (F 14.3, $p < 0.01$) and PH (F 8.9, $p<0.05$).

Table 4.3.1 Values and Standard deviations of Chemical parameters of soil in IRENA botanic garden prior (OUT), during (ON) and post (IN) passage of *C. gambosa* FFRs. F and p-values indicate means equality and significance of the logarithmic transformed data, respectively. Significance is assigned for *p-values* below 0.05

Parameters	IN	ON	OUT	F	*p-value*
Sand	60.6±4.61	62.6±5.03	59.3±9.01	0.2293	*0.801758*
Lime	32±2	25.3±3.05	32.6±7.02	2.6481	*0.149850*
Loam	7.33±3.05	12±2	8±2	2.3188	*0.179440*
CEC cmol*kg-1	34.4±4.14	36.1±1.24	32.2±0.70	1.8550	*0.235936*
Conductivity (dS/m)	0.18±0.08	0.60±0.07	0.11±0.01	**28.5651**	***0.000859***
Hydrophobicity (Sec)	0.79±0.16	26.7±28.9	1.63±0.15	**14.3522**	***0.005168***
Organic matter (%)	12.5±3.02	12.9±3.28	11.0±2.15	0.3473	*0.719915*
Ph	6.79±0.08	6.01±0.30	6.74±0.30	**8.9895**	***0.015666***
K cmol*kg-1	0.37±0.11	0.87±0.29	0.40±0.11	**5.7552**	***0.040231***
NH_4	3.9±0.54	32.8±6.75	4.96±0.85	**123.4859**	***0.000013***
NO_3	4.86±1.76	152.±54.0	17.9±21.7	**13.7129**	***0.005784***
P Olsen (ppm)	18.1±2.93	36.0±17.1	16.3±9.07	3.3600	*0.104953*
Total Nitrogen (%)	0.64±0.08	0.72±0.03	0.59±0.02	4.3553	*0.067852*
B ppm	1.07±0.07	2.51±0.17	1.25±0.19	**53.0584**	***0.000153***
Cu ppm	7.98±2.01	2.91±0.39	9.28±2.23	**21.8917**	***0.001751***
Fe ppm	54.9±18.9	134.±20.7	36.4±8.20	**23.2946**	***0.001485***
Mn ppm	21.2±2.26	111.±5.47	20.8±7.53	**67.1959**	***0.000078***
Zinc ppm	12.6±3.37	2.87±0.55	15.7±2.08	**56.0263**	***0.000131***
Ca cmol*kg-1	25.2±2.22	25.1±2.56	23.5±1.44	0.6285	*0.565178*
Mg cmol*kg-1	4.66±0.35	5.42±0.22	4.37±0.13	**13.0230**	***0.006563***
Na cmol*kg-1	0.13±0.03	0.16±0.02	0.14±0.01	1.5592	*0.284911*
C/N ratio	11.2±1.35	10.3±2.16	10.8±1.74	0.2141	*0.813205*
Ca/mg	5.42±0.21	4.64±0.44	5.39±0.37	4.6278	*0.060837*
K/Mg	0.08±0.01	0.16±0.05	0.09±0.02	4.1911	*0.072605*

Effect of the FFR fungus on telluric bacterial community

Prokaryotic Alpha diversity showed significative variations among the indexes adopted for the analysis (Figure 4.3.1, Table 4.3.2). As generalized effect of the FFR of *C. gambosa*, diversity indexes of bacterial community showed a marked decline in the zone of the active mycelia of *C. gambosa.* In detail, Permanova analysis results evidenced that for bacterial community Shannon index (H') (Pseudo-F 38.24, *p-value 0.011*) and for Pilou's evenness (J') (Pseudo-F 50.348, *p-value 0.029*) undergo to significant changes in the whole transect, while no significative variation are detected when pairwise comparing diversity values between zones of the FFRs (Figure 4.3.1A).

Table 4.3.2 Result of Permutation analysis of variance (PERMANOVA) on changes in alpha diversity indexes of prokaryotic community within FFR zones and results of PERMANOVA tests on pairwise comparisons between C. *gambosa* FFRs zones. pseudo-F and t values explain mean equality. Significance of *p-values* below 0.005 is showed in bold.

	Number of OTU		H'		J'	
	pseudo-F/t	***p-values***	***pseudo-F/t***	***p-values***	***pseudo-F/t***	***p-values***
FFR zones	13.823	*0.193*	**38.249**	***0.011***	**50.348**	***0.029***
OUT vs ON	11.325	*0.315*	18.764	*0.095*	21.718	*0.092*
ON vs IN	12.569	*0.202*	20.413	*0.097*	23.807	*0.105*
IN sv OUT	0.343	*0.915*	12.604	*0.308*	0.725	*0.596*

More evident variations are observed at phylum levels between sampling zones of the FFR. In more detail, the zones characterized by the presence of the active mycelium of *C. gambosa* promote the increase of *Proteobacteria* and *Bacterioidetes* at the expense of *Actinobacteria, Verrucomicrobia, Plantomyces* and *Acidobacteria*. Notably, among these phyla *Bacterioidetes* showed the higher level of fluctuation passing from 9% of relative abundance in the OUT soil until 27% of the total community in the ON zones. Finally, abundance of *Bacterioidetes* restore at level similar to those present in the soil previous the passage of *C. gambosa* (8% in IN zones). Similar behaviour but with a less extensive effect is noticed for *Proteobacteria*, passing from 39% of relative abundance in OUT to 49% in ON and restoring at 35% in IN (Figure 3.3.1B).

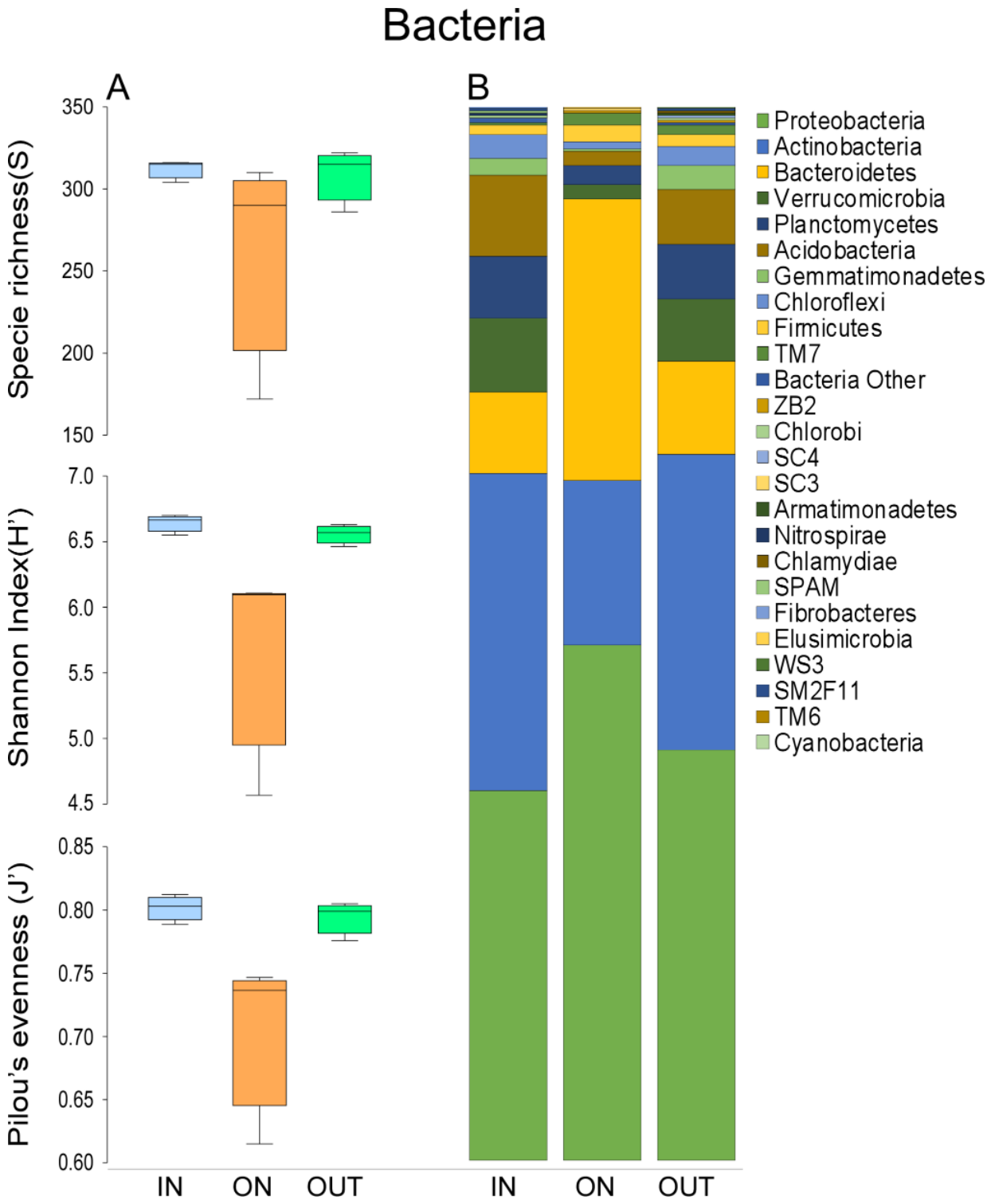

Figure 4.3.1 A) box plots of bacterial diversity in the three zones of the FFR (IN, ON, OUT). Alpha diversity is expressed as Species richness (OUT's number), Shannon index (H') and Pilou's evenness (J'). B) bar plot of relative abundance of the main prokaryotic phyla in the three FFR zones

Contribution of single bacterial Taxa are showed in heatmap. Changes in community are showed according to hierarchical clustering based on Bray-Curtis similarity for the samples and index of association for variables (Figure 4.3.2). Ordination of FRs zones showed an earlier segregation for ON3 sample with higher level of dissimilarity with the other sampling zones (segregation at 21.31 values of Bray-Curtis distance. *p*-

value 0.001). Other cluster formation event is assessed at 52,16 level of similarity (*p-value 0.001*) inducing the formation of two defined cluster including the sampling zones correspondent to the active mycelium of *C. gambosa* and the OUT2 zone. Other sampling zones external and internal to FFRs clustered at 69.26 of Bray Curtis similarity values (*p-value 0.001*). Correspondingly Bray-Curtis ordination of sampling zones is operated by specific bacterial taxa. First cluster composed by ON3 zone is characterized by the higher presence of *Sphingobacterium, Parapedobacter* and *Tetrathiobacter*. The Second cluster is formed by sampling zones of the active mycelia of *C. gambosa* and the main contributing taxa are *Sphingobacteriaceae*, *Sphingomonas, Streptomyces, Pedobacter, Devosia and Rhodanobacter.* Other sampling zones, internal and external to the FR are characterized by a homogenously distributed bacterial community only dominated by the presence of *Pseudomonas* genus that is however common also in sampling zones characterized by the mycelium activity of *C. gambosa* (Figure 4.3.2).

Qualitative assessment of bacterial turnover-overlap is visualized in Venn diagram (Figure 4.3.3). The analysis evidenced that core microbiome is composed by 308 taxa (commonly found in all the zone of the FRs). Starting by OUT zone, 13 taxa were exclusively found externally to the FRs. These are substituted by 16 exclusive bacterial taxa getting advantage by the conditions imposed by *C. gambosa* activity with an average overlap of 32 taxa between the OUT and ON zones. In the same extent, the end of the activity of the FR fungus promote the instauration of 12 exclusive taxa inside the ring (IN) with an overlap of 23 common taxa between the adjacent sampling zones. Notably, overlap of non-contiguous zones produce the higher level of bipartite overlap (61 taxa).

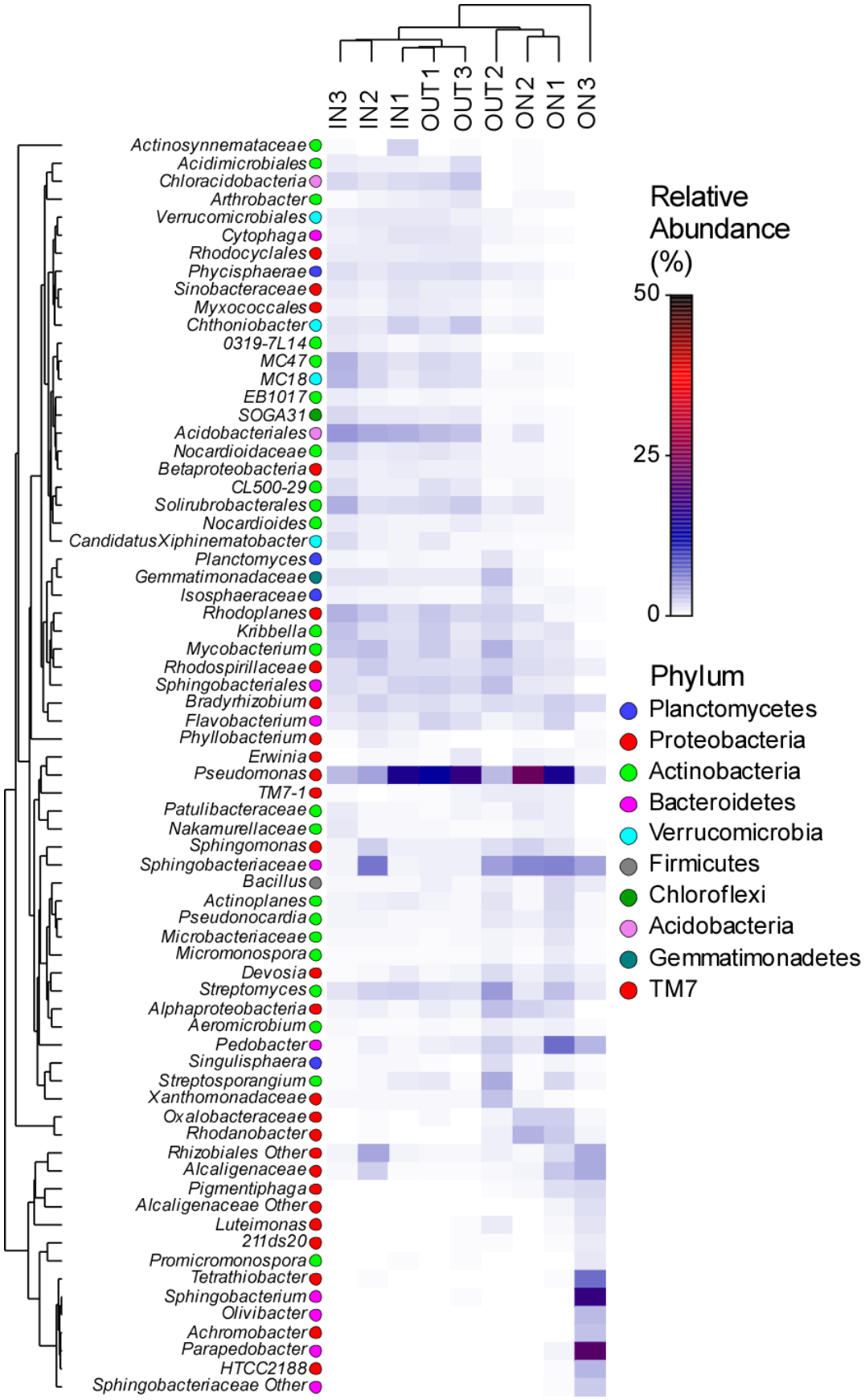

Figure 4.3.2: heat map of prokaryotic relative abundance in the three FFR zones. Hierarchical ordination of samples and variables are made according to Bray Curtis similarity and association index, respectively. Variable are selected according to the 50 most abundant taxa in the dataset. Taxonomy is assigned at the least taxonomical level.

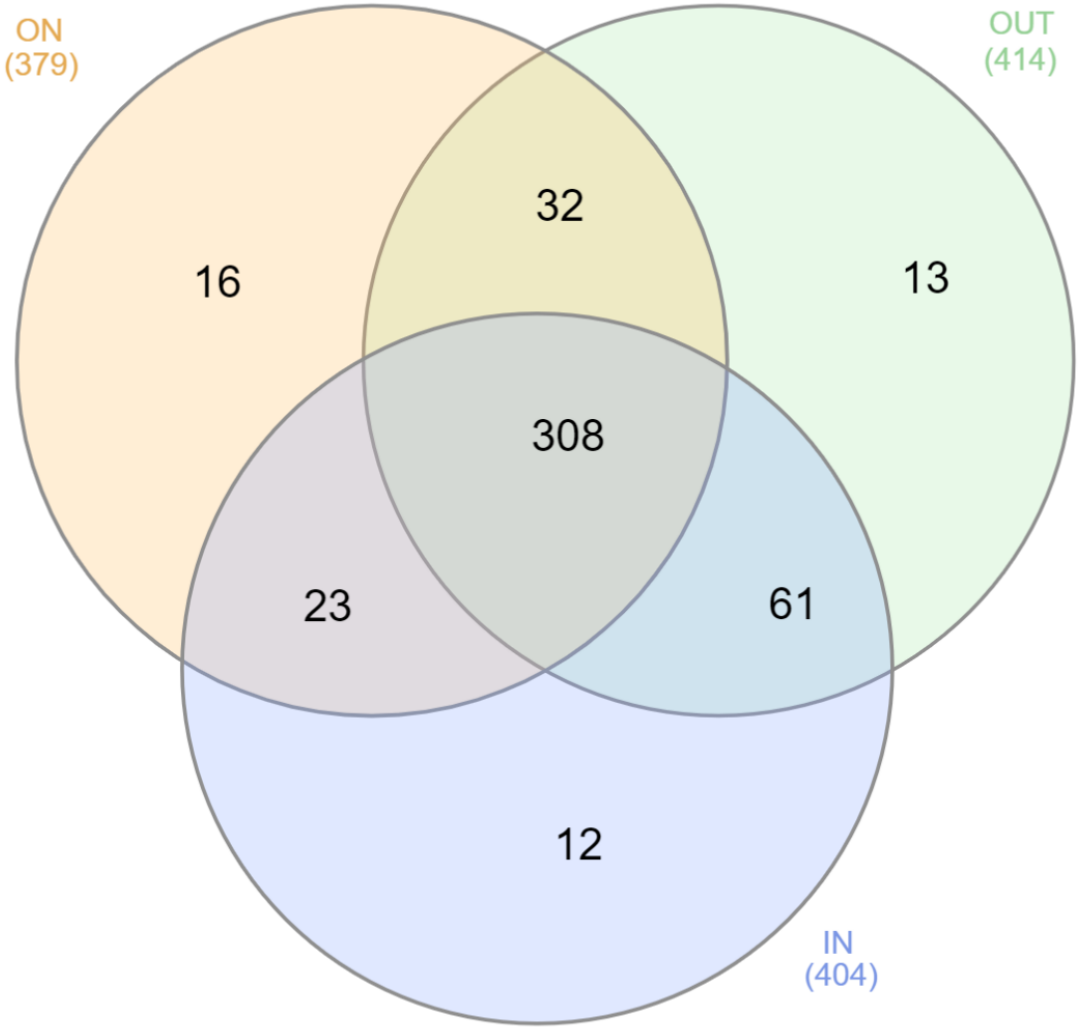

Figure 4.3.3 Ven diagram showing overlapping and exclusive prokaryotic microbiome taxa in the three FFR zones.

Effect of the FR fungus on telluric mycobiota

Alpha diversity of mycobiota showed significative variations among the indexes adopted for the analysis (Figure 4.3.4, Table 4.3.3). The index of diversity of fungal community undergo to an overall decrease in the zones marked by the presence of the FR fungus. With more detail, Permanova analysis results evidenced significant changes for the number of operational taxonomical units (OTU) in the transect across FRs (Pseudo-F 11.75, *p-value 0.017*). Oppositely to what described for bacterial community, significative variations are detected when pairwise comparing Shannon index values between zones of the FRs (ON vs IN) (t 33,26, *p-value 0.036*) (Figure 4.3.4A). At phylum levels differentiation among FR zones are mainly due by the increased presence of *Basidiomycota* (77.1 % of relative abundance) at the expense of all the other fungal phyla (Figure 4.3.4B).

Table 4.3.3 Result of Permutation analysis of variance (PERMANOVA) on changes in alpha diversity indexes of fungal community within FFR zones and results of PERMANOVA tests on pairwise comparisons between C. *gambosa* FFRs zones. pseudo-F and t values explain mean equality. Significance of *p-values* below 0.005 is showed in bold.

	Number of OTU		H'		J'	
	pseudo-F/t	***p-values***	***pseudo-F/t***	***p-values***	***pseudo-F/t***	***p-values***
FFR Zones	**11.754**	***0.017***	43.181	*0.118*	33.621	*0.163*
OUT vs ON	35.165	*0.113*	22.867	*0.098*	20.063	*0.112*
ON vs IN	55.595	*0.096*	**33.265**	***0.036***	2.784	*0.058*
IN vs OUT	0.741	*0.484*	0.245	*0.798*	0.188	*0.856*

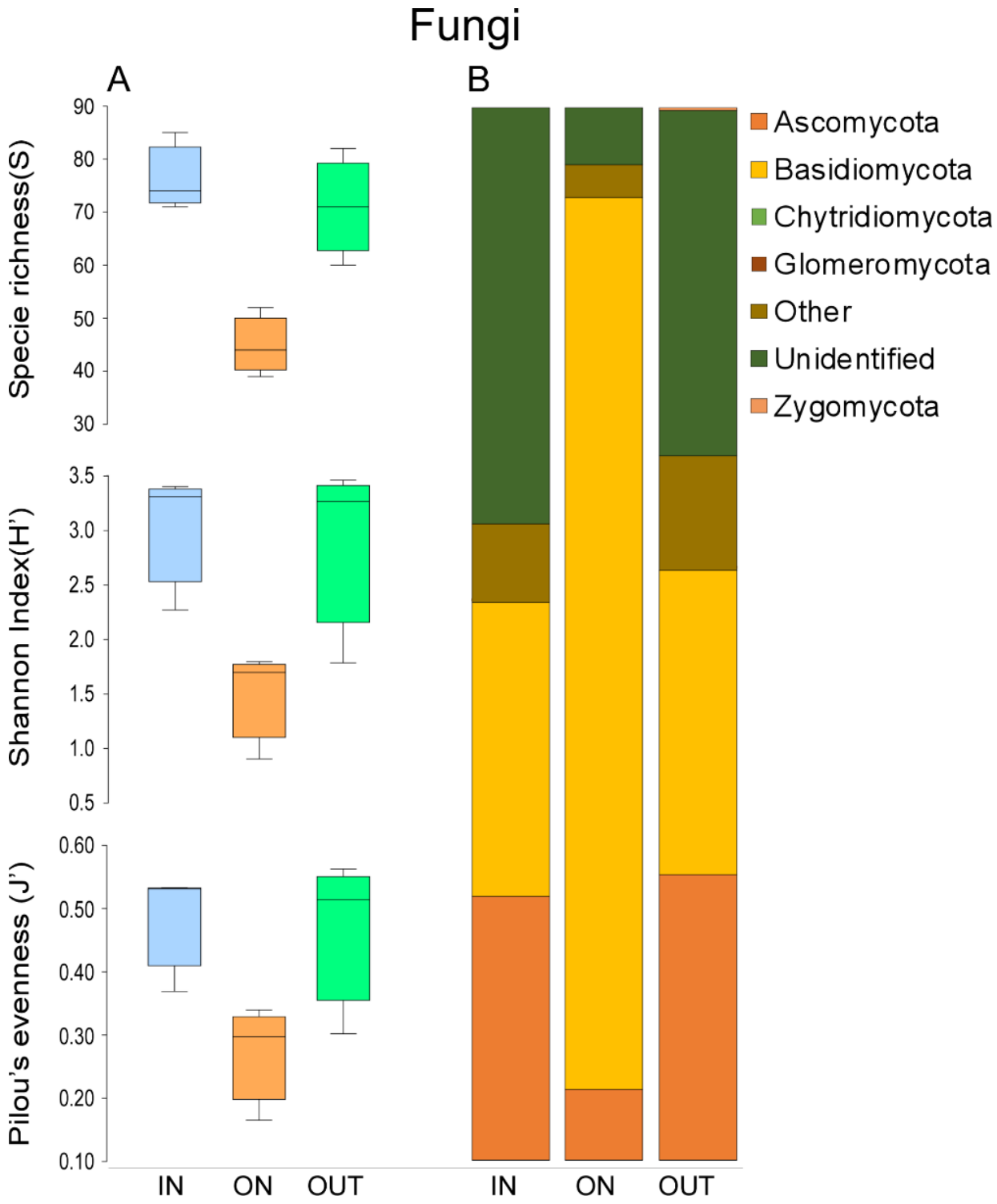

Figure 4.3.4 A) box plot of mycobiota diversity in the three zones of the FFR (IN, ON, Out). Alpha diversity is expressed as Species richness (OUT's number), Shannon index (H') and Pilou's evenness (J'). B) bar plot of relative abundance of the main prokaryotic phyla in the three FFR zones

As expected, heatmaps of best seventy taxa in fungal community evidenced that changes in *Basidiomycota* abundance is given by the dominant presence of the FR forming fungus *C. gambosa* (Average relative abundance of 82.2%). Concomitantly with the active presence of the FR fungus a generalized simplification

of the fungal community taxa is observed. The increase in abundance promote segregation of a cluster composed exclusively by the ON zone samples according to Bray Curtis distance values (14.21, *p-value 0.002*), Interestingly a single segregation is formed when comparing community data of OUT 2 sampling zone, showing another fungal dominance operated by a *Basidiomycetes* belonging to *Lepiota* genus. Notably the fungal communities sampled in the present experiment showed other case of fungal dominance operated in conjunction of *Lepista* and *Gymnopus* Basidiomycotan genus. The sampling zones not interested by the effect of *C. gambosa* are characterized by the higher presence of unidentified fungi and unidentified *Ascomycota* (Figure 4.3.5).

Turnover-overlap in fungal community is visualized in Venn diagram (Figure 4.3.6). Core mycobiota is composed by 57 taxa. With FRs passage the OUT zone lost 22 exclusive taxa and overlap in the shift from OUT ot ON zones by only 2 fungal OTUs. The ON zones became characterized by 6 taxa and overlap with the subsequent zone by 5 OTUs. Following the effect of *C. gambosa* the IN zone became characterized by 20 taxa and, as showed also for bacterial community, the higher level of bipartite overlap is between the IN and OUT zone sharing (26 taxa).

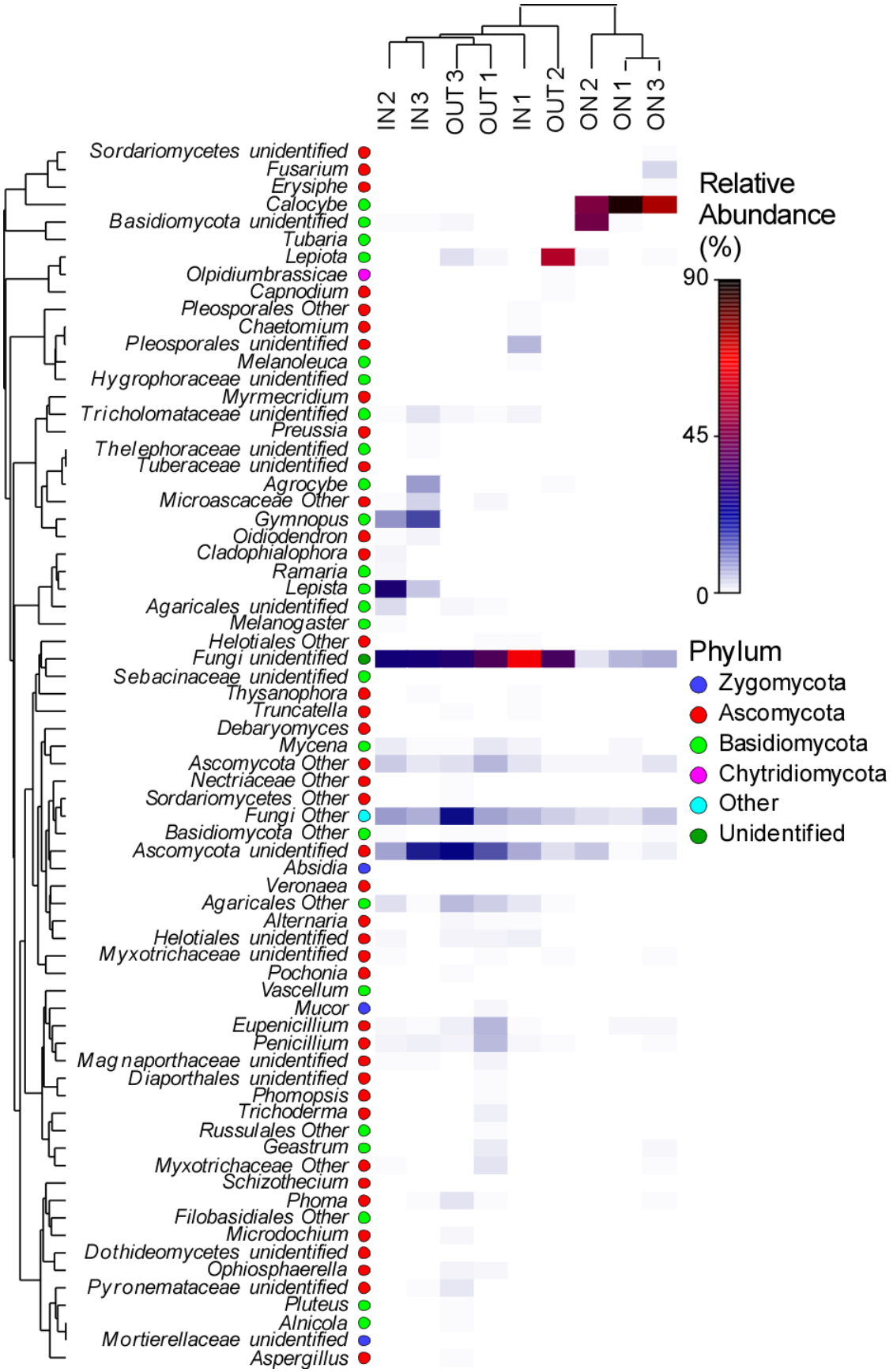

Figure 4.3.5 heat map of mycobiota relative abundance in the three FFR zones. Hierarchical ordination of samples and variables are made according to Bray Curtis similarity and association index, respectively. Variable are selected according to the 50 most abundant taxa in the dataset. Taxonomy is assigned at the least taxonomical level.

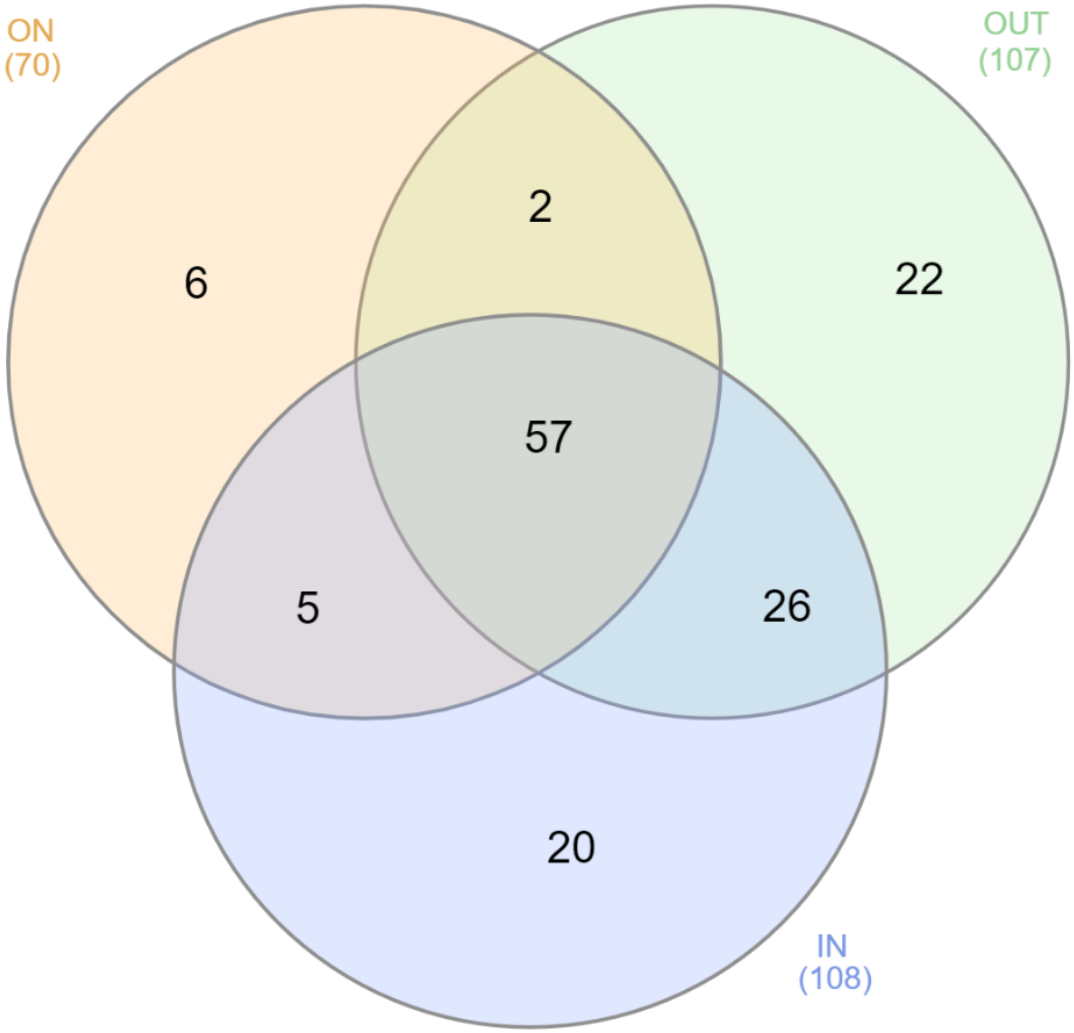

Figure 4.3.6 Ven diagram showing overlapping and exclusive fungal taxa in the three FFR zones.

Soil Physicochemical changes triggered by C. gambosa FFR promote a cascade of interaction in telluric microbiota.

Sampling points were plotted in CCA to test the interactive effect of *C. gambosa* with the surrounding telluric microbiota (Figures 4.3.7A, 4.3.8A). Additionally, main contribution of physicochemical characteristics was associated with bidimensional disposition of different communities in FRs zones (Figure 4.3.7B, 4.3.8B). From the analysis, the abiotic variables were filtered according the most effective multiple correlation values. For both bacterial and fungal community the CCA analysis evidenced a segregation of the sampling points with the massive presence of *C. gambosa* mycelium respect to those inside and outside the FR. However, some discrepancies in clustering of the sampling zones is observed for bacterial community in sample ON3 and sample OUT1. Commonly for soil microbiota in our study transect, the different community structuration according to the action of the FRs are positively associated with the fluctuation in Hydrophobicity, Mn, NO_3

and Fe while are with less extent negatively correlated with organic matter. Specifically, for bacterial community, positive association with community changes are slightly dependent by NH_4 content in soil, meanwhile for fungal community, level of P is considerably included in the most effective soil chemical variables. Filtering of soil physicochemical parameters provide data reduction to assess the intrinsic variability characterizing the different communities. Heatmap generated from Spearman Rank correlation values evidenced that both the communities have a dissociated effect between soil parameters and organic matter content as evidenced by Euclidean distance clustering (Figure 4.3.7C and 4.3.8C). Specifically, for bacterial community the cluster formed by the physicochemical parameters indicate a covariable behaviour of the seventy taxa in the original communities. Moreover, is quite clear the division of the bacterial community because of positive and negative values of Spearman rank correlations.

Oppositely for the seventy fungal taxa the Euclidean distance clustering showed internal segregation in the effect of physicochemical parameters on mycobiota. The communities differently respond to higher level of NO_3 and hydrophobicity and increase of Fe, P and Mn. Interestingly, few fungal taxa respond positively to fluctuation of organic matter leading to the formation of four main groups that responds to the variations induced by *C. gambosa.*

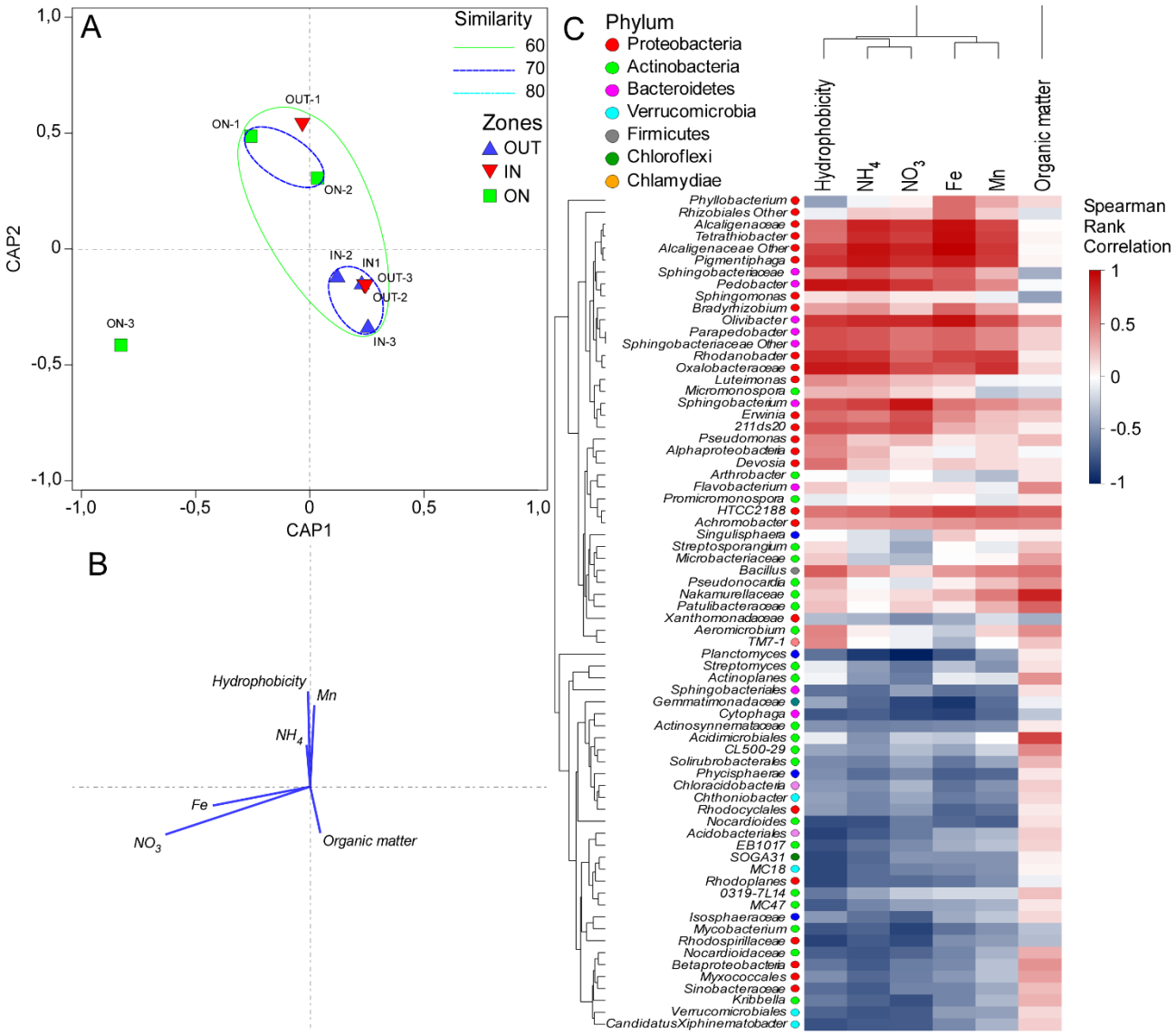

Figure 4.3.7 a) loading plot of CCA (Canonical correspondence analysis) ordination of prokaryotic communities in the FFR zones. ellipse indicate similarity clustering based on Bray Curtis similarity metrics. b) score plot showing main multiple regression association of prokaryotic community variation with soil parameters un the three FFR zones. c) heat map of Spearman Rank correlation between soil chemical parameters screened from CCA and best 50 prokaryotic taxa. Hierarchical ordination of sample was made according to Euclidean distance, while ordination of variable is made by association index.

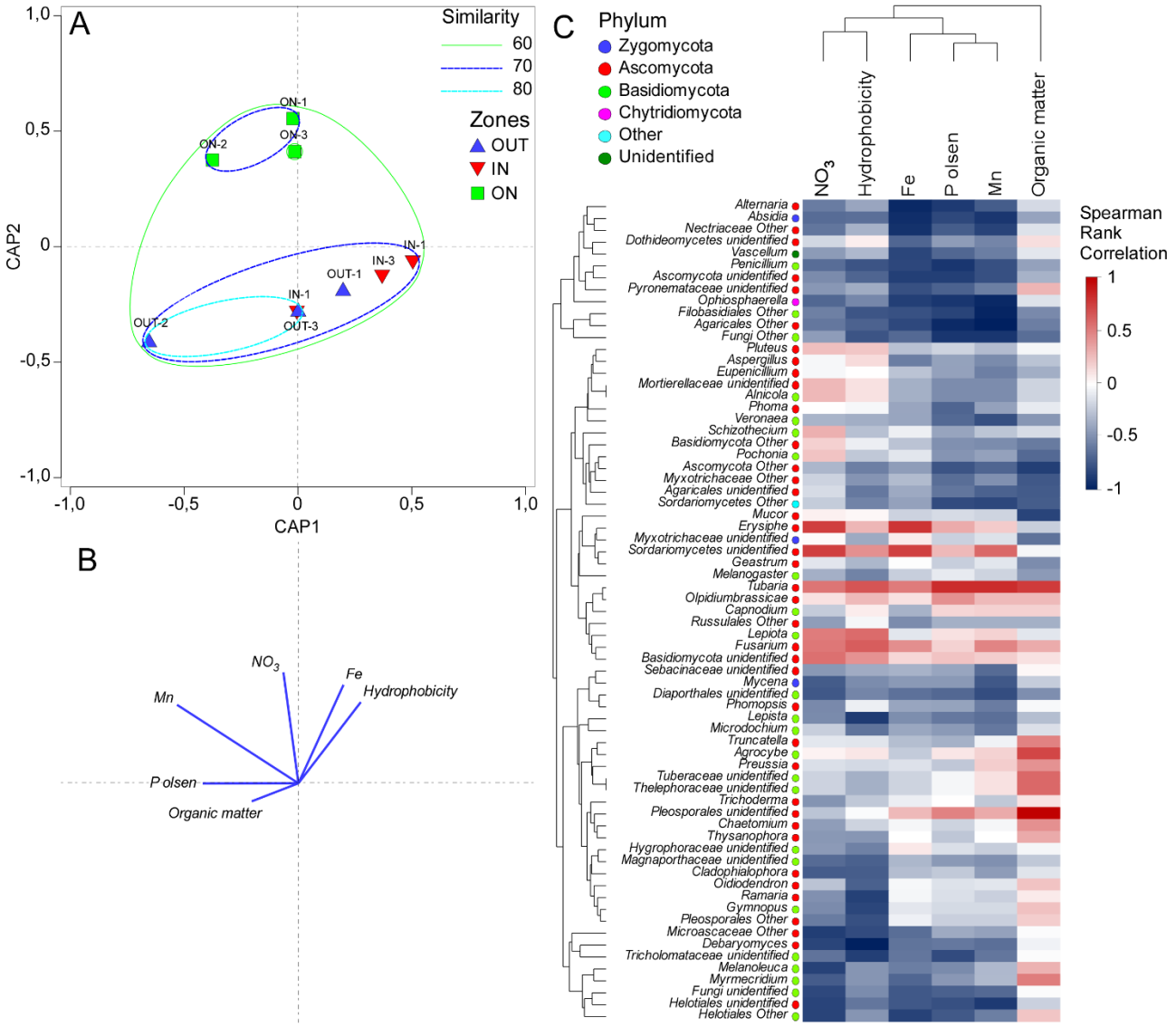

Figure 4.3.8 loading plot of CCA (Canonical correspondence analysis) ordination of fungal communities in the FFR zones. ellipse indicate similarity clustering based on Bray Curtis similarity metrics. b) score plot showing main multiple regression association of prokaryotic community variation with soil parameters un the three FFR zones. c) heat map of Spearman Rank correlation between soil chemical parameters screened from CCA and best 50 fungal taxa. Hierarchical ordination of sample was made according to Euclidean distance, while ordination of variable is made by association index.

By general point of view, qualitative comparison of the effect promoted FRs on both bacterial and fungal community network, based on Spearman Rank correlation data, showed an evident suppressive effect by the activity of *C. gambosa* when compared to the number of the positive interactions observed. when comparing the number of positive and negative interaction for each physicochemical parameter, the generalized tendency to produce higher number of negative interactions is confirmed for all the variable in exception for relation between organic matter and bacterial community with a positive delta. Finally, comparing the number of negative and positive interaction across the two-community subject to the passage of *C. gambosa* is observed that bacterial community is more capable to produce positive interaction compared to the fungal community.

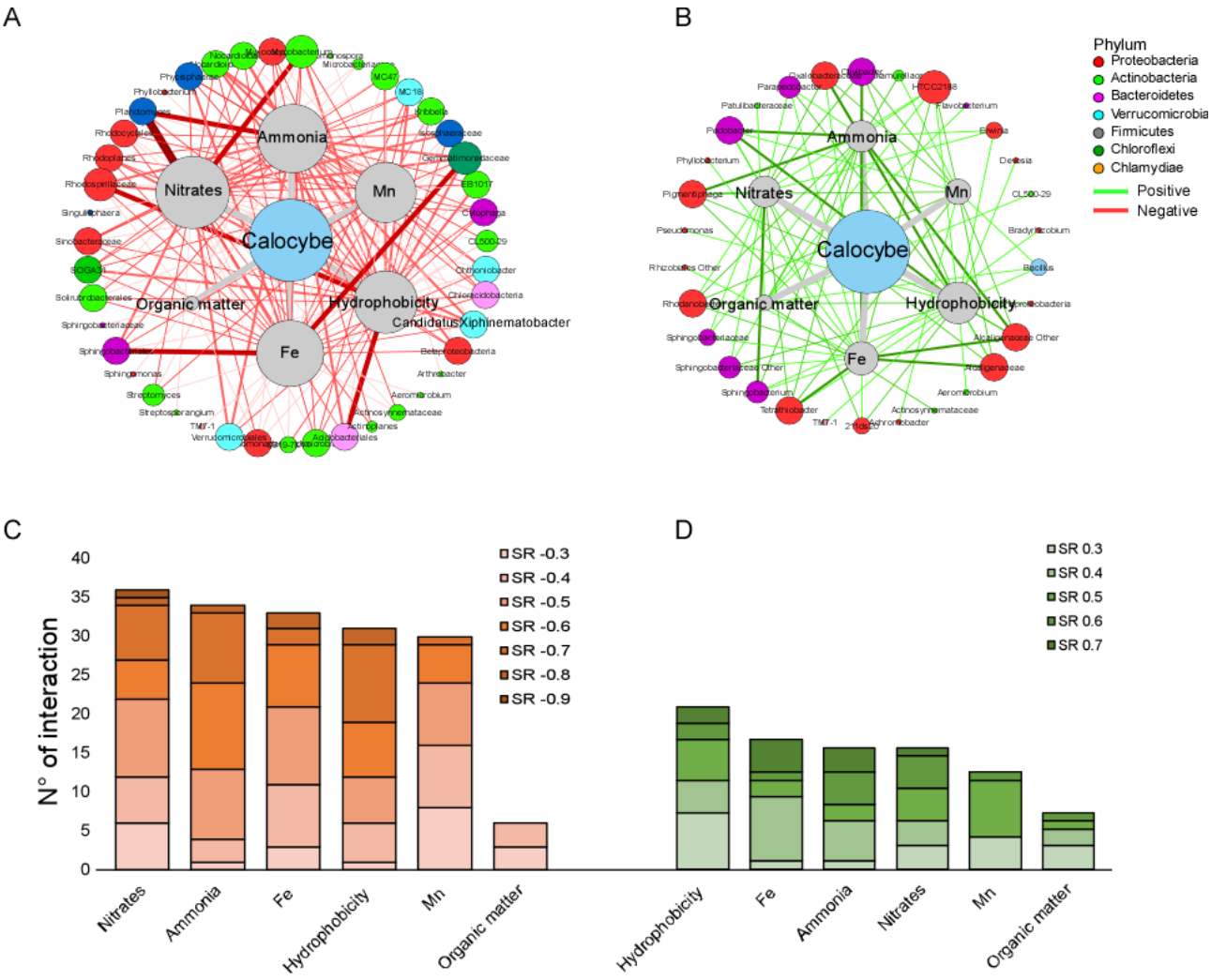

Figure 4.3.9 A and B) Network of interaction explaining the changes imposed by the FFR fungus C. gambosa and in turn how it affects the whole bacterial community. A) shows negative correlations and B shows the negative one. Edge thickness is log proportional to spearman correlation values. C and D) bar plots showing the number and strength of interactions produced by *C. gambosa* on the physical chemical characteristics of soils in the three zones of the FFR.

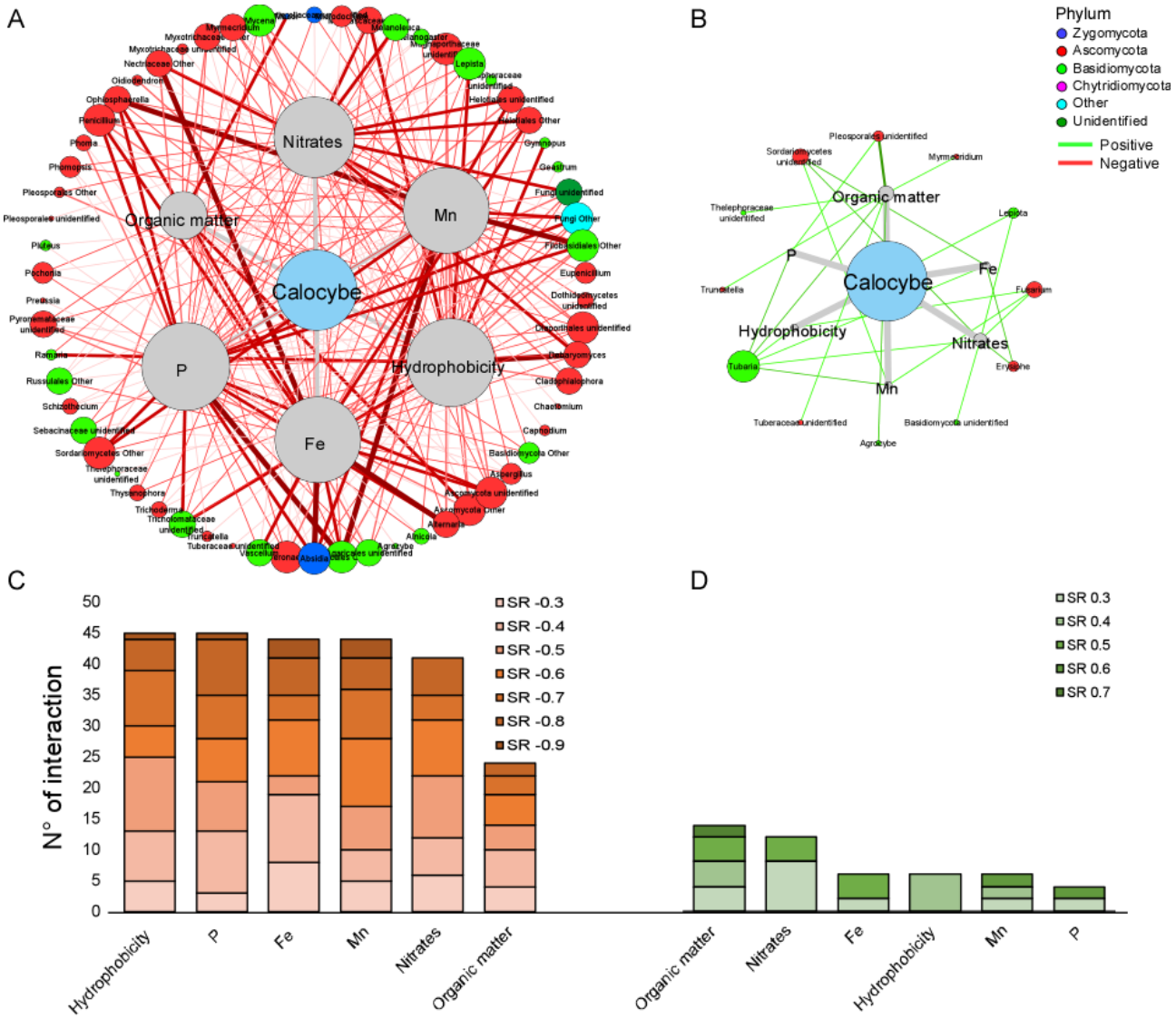

Figure 4.3.10 A and B) Network of interaction explaining the changes imposed by the FFR fungus C. gambosa and in turn how it affects the whole fungal community. A) shows negative correlations and B shows the negative ones. Edge thickness is log proportional to spearman correlation values. C and D) bar plots showing the number and strength of interactions produced by C. gambiosa on the physico chemical characteristics of soils in the three zones of the FFR.

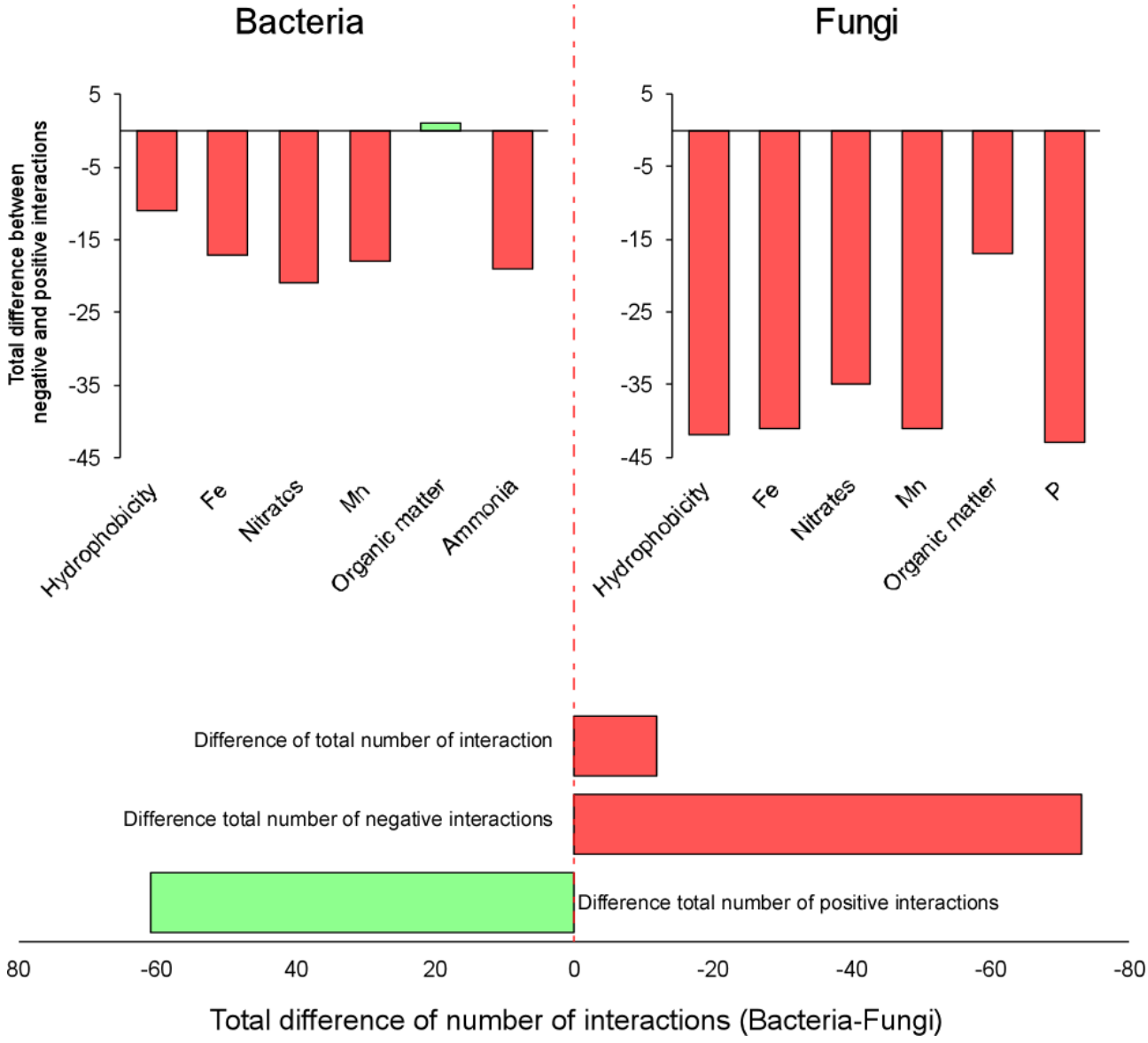

Figure 4.3.11 difference in number of positive and negative interactions for bacterial and fungal community and difference in number of positive, negative and total number of interactions between bacterial and fungal community.

4.3.4. Discussion

In the present work we described the effect of a Type I FR of *C. gambosa* on both bacterial and fungal community in soil. Our results are in accordance with previous work describing the effect of FR on physico-chemical and microbial properties of affected soil (Gramss *et al.*, 2005; Bonanomi *et al.*, 2012; Espeland *et al.*, 2013). The physicochemical parameters included into the study showed changes in soil characterized by active mycelium of *C. gambosa,* although a part of those, did not change enough to be considered significative and is maybe given by the limited number of samples collected.

Interestingly, when comparing bacterial and fungal communities, the fluctuations due to the passage of FR triggers two distinct behaviour in each community counterparts. Indeed, bacterial community showed e sharp division in taxon. With one getting advantaged from the physicochemical changes apported by the fungus and another portion, almost of the same magnitude of taxa, being supressed. Inversely, mycobiota face a

generalized simplification showing a more articulated response to fluctuation of physicochemical parameters in soil. As general view, is possible to assess that bacterial community developed an increased resilience to environmental fluctuation with few taxa specialized to get advantage in hydrophobic conditions or used chemicals released as optimal substrate for metabolic processes. On the other part, the simplification of the mycobiota suggest that fungi are more responsive to different environmental changes, do not showing specific increase in taxa abundance coupled with the variation triggered by the dominant *C. gambosa*.

With more detail, the positive changes in NH_4^+ operated by the fungus has been extensively reported in previous works describing the effect of the FRs. This recurrent fluctuation indicates that the effect triggered by saprobic activities of the Basidiomycete is a conserved trait at interspecific level and extend the chemo-ecological groups of Ammonia fungi (Sagara, 1975). In mechanistic terms, The effect in enrichment of Ammonia could be considered as a by-products of decomposition processes in the FR but the total amount of ammonia could be also considered as a product of nitrates dissimilatory/assimilatory reduction pathway that is common for *Sphingobacteriaceae* (Steyn *et al.*, 1998) and *Fusarium* (Takasaki *et al.*, 2004) being among the few bacterial and fungal species advantaged from the passage of the FR. This make it difficult to discerns whether the production of ammonia is majorly promoted by the action of *C. gambosa* or the biological ability of these few bacterial taxa selected in the FR zone. However, is possible to hypothesize a cyclic pattern in consumption/generation of NH_4 . Bacterial and fungi can transform NH_4 produced by the decomposition of organic matter in NO_3 that in turn can be reconverted to NH_4. The cyclic transformation of nutrients could be considered taking place until substrates for the cycle are lost by leaching given the high motility of NO_3 in soil (Davidson *et al.*, 1992; Rütting *et al.*, 2011). However, whether on one side the presence of NH_4 is crucial for the determination of how the bacterial community is shaped, these levels are not significantly considered for fungal community that are more negatively related to the presence of NO_3 coupled with increased Hydrophobicity in soil. The same behaviour of the two parameters indicates as well that NO_3 production is more related to the presence of fungal biomass compared to those of NH_4, being hydrophobicity a direct effect of the presence of the fungal mycelium of a dominant decomposer fungus (Rillig, 2005).

We also observed that in the active zones of *C. gambosa* an increase in values for soil elements as often take place for the members of the Basidiomycota phylum (Dighton, 2007). Enrichment on elements are fundamental for saprobic Basidiomycetes as they are used as co-factors in zymogenous processes, formulation of specific secondary metabolites or in the fine regulated process of biomolecules catabolism (Baldrian, 2003). Particularly, is observed a decoupled effect of Fe, Mn and P on mycobiota, indicating that the causative agents of the community simplification are different, although the whole fungal community is suppressed. For Fe the increase observed in correspondence of the mycelium is probably related to the carbohydrate consumption (Howard, 1999). Indeed iron is directly involved in utilization of atmospheric nitrogen, the synthesis of deoxyribonucleotides, respiration, the tricarboxylic acid cycle, and the synthesis of numerous small molecules such as amino acids, lipids and sterols (Philpott, 2006). Moreover is a fundamental element homeostatic regulation in fungi (Kosman, 2003). Distinct requirement of metallic ions is inversely observed for Mn. Particularly is acknowledged that Mn is fundamental in oxidative processes to regulate enzymatic activities in organic matter

decomposition (Bonnarme & Jeffries, 1990; Santelli *et al.*, 2011). Finally, the high enrichment of P is probably due to the endogenous requirement of the FR-fungus in order to carry over the high magnitude of catabolic and biosynthetic processes involved in a pattern of monodominance. Taking in account these suggestions is possible to argue that the microbial communities in soil, and particularly a portion of the fungal community, is out-competed by the action of the fungus and the unavailability of certain compounds for the other microbial counterparts. Indeed, fluctuation of Fe, Mn an P are related to the requirement of the fungus that are immobilized in different way, like formation of Fe-S granules in the extracellular matrix from homeostatic regulation of the fungus or are not available in soil because bonded internally to the fungal mycelium.

By the point of view of the strategic adaptation of the FR fungus, *C. gambosa* showed to act at different level in order to exclude other microbial population present in soil. This behaviour is characteristic of Basidiomycetes being combative fungus (C-selected). These fungus are characterized by slow-growth and the ability to produce a series of environmental modification act to promote its own dominance in order to monopolize resources (Pugh, 1980; Dix, 2012). To date the out-competition of microbial community by a fungus could be operated in direct and indirect way (Deacon, 2013). In our survey we observed the action of modification of the soil physicochemical parameters that could be included as indirect interactions with the soil microbiome. Immobilization of fundamental cofactors for the advancement of metabolic and catabolic pathway in a community and instauration of drought condition are suitable to be considered the most important drivers promoting simplification in soil microbiota. However, the direct effect of the acknowledged ability of Basidiomycetes to release antibiotic compounds in soil could not be excluded, indicating that the understanding of the mechanisms behind FR effects and action still require extensive studied and meticulous experimental planning. Nevertheless, some intriguing hypothesis could be formulated according to some of the results obtained in the present work. Therefore, the particular and ambiguous condition in which FR acts lead us to think that FR fungus do not exclusively act as detrimental agent for soil microbial community. This because of the increased population of specific bacterial taxa take advantage in the conditioning effect of the fungus. In fact, in some work centred on FR metagenomics the bacterial community is simplified but preserve some selected taxa. These particular cases could be considered as effect of mycoparasitism as in the case of *Burkholderia* and *A. arvensis* (Zotti Unpublished) of bacterial but endosymbiotic relation between FR-forming fungus and bacteria could not be excluded (Schimel *et al.*, 1999; Sardans & Peñuelas, 2005). Interestingly the mismatching between *Sphingobacteriaceae* relative abundance and hydrophobicity induced in soil indicate that those bacteria obtain water for maintaining proper level of cellular osmolarity by the only place where it is available that is, in this case, the mycelium of FR forming fungus *C. gambosa.*

4.3.5. Conclusions

Our results, are largely centred on microbial community in soil and the effect of *C. gambosa* on it. However, we observed effect of the fungus in the vegetation persisting on the FR (Data not showed) including the FRs of *C. gambosa* in this study belong to Type I. In our opinion the common factor promoting both plant death, and simplification of soil microbial community is the increase in hydrophobicity in soil. Several authors hypothesize that the effect could be caused by pathogenic behaviour of the fungus or the release of phytotoxic compounds. To date we are not able to exclude these hypotheses because we were not able to measure these kinds of parameters in soil. However, the FRs studied in this work showed in front and behind the zone with the active mycelium some narrow strips of stimulated vegetations similarly to those of *M. oreades* studied by Hardwick and Cosby in in 1978 and 1960. This appear to be in discordance to both the hypotheses but in accordance to studies carried out on "Fairy chemicals" produced by *Lepista sordida* and promotive of vegetation rather than detrimental (Choi, JH *et al.*, 2010). Despite the mismatch, we are in accordance with some of that works including FR as important phenomenon in ecosystem processes and functioning (Bonanomi *et al.*, 2012; Yang *et al.*, 2019). The destabilization effect produced by the fungus could be considered as study model for explain importance of biological disturbances in ecosystem functioning by point of view of community ecology and species richness patterns but also as fundamental driver for advancement of biogeochemical processes.

To date, the present work with our interpretation is limited by the number of samples and the dependency on just one FR species. In order to further assess, the importance of FR as ecosystem engineer in terrestrial environment more extensive studies are required with the possibility to rely on inter-ecosystemic and interspecific information.

4.4. On the mechanisms of Plant-Fairy ring fungus interaction: a microbiological assessment of VOCs release.

4.4.1. Introduction

Fungal Fairy Rings (FR) are important phenomenon in terrestrial ecosystem. A series of study reported it as fundamental agent in structuring plant communities (Zotti unpublished) (Bonanomi *et al.*, 2012) and also affecting soil microbial composition (Ohara & Hamada, 1967; Kim *et al.*, 2013; Oh & Lim, 2018b). Particularly FR action produce different outcome on vegetation, primarily depending if it belong to grassland or woodland ecosystem and secondary depending by FR forming fungus species (Yang *et al.*, 2019). Focusing on grassland, the outcome of FR action may be grouped in three main typology according to the effect on vegetation (Shantz & Piemeisel, 1917). Within the Type 1 FRs are included those fungi producing a detrimental effect combined with a promotive one. Type 2 FRs only produce apparent positive effect on vegetation, while Type 3 have no effects.

To date the most studied FR are those from type 1 and 2 (Zotti unpublished review) where detrimental ad promotive effect on vegetation fascinating ecologist in light to explain ecosystem functioning pathway. As consequence, different studies succeeded with the aim to disentangle the mechanisms behind the action of FR on grassland vegetation. Several hypotheses were formulated according to scattered or incomplete evidences. Therefore, some studies explained the detrimental effect of FRs as an increase of Hydrophobicity because the release of hydrophobines by denser mycelium of the FR fungus (Gramss *et al.*, 2005). These studies also suggest that higher is the mass of mycelium in the soil and higher is the probability to forms Type 1 FR (Shantz & Piemeisel, 1917). Other works tried to explain the pattern through evidence of formation of pathogenic haustoria like structure from *Marasmius oreades* (Elliott, 1926). The same fungus was inquired to kill vegetation through a production of cyanohydrins compounds (Blenis *et al.*, 2004).

To date no discriminant evidence are present in modern bibliography in order to support each of these hypotheses. Therefore, of the direct interaction between FR fungus and plants and the ecological repercussion of this interaction remain unsolved. Interestingly, in the last ten years direct application of liquid extracts from the FR fungus *Lepista sordida* was tested with surprising results of an enhanced growth of plants treated (Choi, JH *et al.*, 2010; Choi *et al.*, 2014). However the results obtained was more efficiently used in order to promote a promising application in agriculture instead to furnish fundamental ecological information in light of the importance of the FR fungus as ecosystem engineer (Bonanomi *et al.*, 2012; Kawagishi, 2018). Additionally, a significant scientific effort was acknowledged being carried out for the scientists involved in study of this mechanism in *L. sordida*. Indeed, recently the whole genome of the fungus has been sequenced as it is a topic with promising economic and scientific purposes (Takano *et al.*, 2019). Unfortunately, the works regarding ICA and AHX growth regulator for FR fungus applied extract rather than uses direct application of the fungal colony.

To obtain crucial information on the mechanisms behind FR action and direct interaction with plants we planned and experiment with isolates of type 1 FR *Calocybe gambosa.* We tested the potential effect of production of cyanohydrins as detrimental factor promoting the formation of a belt with poor or absent

vegetation. Particularly, given the volatile nature of cyanohydrins (Park *et al.*, 2004) we tested the effect of VOC emitted by the fungal isolate of *C. gambosa.*

4.4.2. Materials and methods

Fungal material collection and isolation.

Fungal carpophores from three regular shaped FR of *C. gambosa* was collected in May of 2019 in the botanical garden of Escuela Superior y Técnica de Ingeniería Agraria of León University. Rings was recognized belonging to type 1 FR by the combined detrimental and stimulating effect on vegetation. Biological samples were carefully cleaned from soil detritus and carefully placed in sterile sealed bags. Sample was promptly transported in laboratories of IRENA (Istituto de REcursos NAturales) in León University and fungus recognized at species level by characteristic morphological feature and shapes of fruiting bodies.

In order to obtain pure culture of *C. gambosa,* carpophores were surface sterilized by immersion in sodium hypochlorite at 4 % concentration for a period of 10 minutes. Following, sterilized carpophores were placed in sterile hood and surface dried. Inoculum of vegetative mycelium of *C. gambosa* were dissected from internal portion of fungal carpophores and placed in Modified Melin-Norkrans medium (MNM) previously poured in petri dish of 9 mm and solidified in sterile hood. Culture media and methodology are prepared according to (Marx, 1969; Angelini *et al.*, 2012). Inoculated petri dish was sealed with parafilm and cultivated for 2 weeks at room temperature in dark conditions. After growth period plates was screened for contamination and those presenting anomalous colony or translucent surfaces was eliminated from the experiment.

VOC bioassays

We planned 2 experiments in order to determine effect of VOC release from *C. gambosa* on plants. As test plant species we used *Triticum estivum* because the fast-growing and annual life strategy of the species. Seeds of *T. estivum* was previously sterilized by immersion in ethanol 70% for 1 minute and KOH 4% for ten minutes. As general methodology we used the petri dish sandwich method (Dennis & Webster, 1971). In detail, in order to permits passage of VOCs we excluded Petri dishes caps. Following, Petri dishes containing the fungus and seeds were put in contact by overlapping them. In order to avoid changes in seed disposition, petri containing seeds was placed beneath that containing the fungal colony of *C. gambosa*. Additionally, seeds were gently fixed in water agar, previously autoclaved, poured and cooled in petri dishes. Between the two petri dishes containing fungus and seeds a sterilized dialysis membrane was placed in order to avoid possible contamination of the mediums.

We compared the effect of the FR-fungus by recording seed germination in experimental systems with the presence and absence of an active colony of *C. gambosa.* In control system germination was recorded in a subset of seeds against petri dishes without *C. gambosa* colony but only in the presence of Modified Melin-Norkrans medium. The first experiment was a preliminary test of comparison of germination in presence and absence of the first isolates of the fungus. In the second assay, we used a different approach in order to check whether the effect observed in the first experiment was related to the presence of the fungus or is dependent

by aleatory factors. In order to check the strength of the interaction, the second experiment was performed with higher number of replication (five for each treatment) and consists in application of petri inoculated with 1, 2 and 4 plugs of *C. gambosa* against five petri dishes containing seven *T. estivum* seeds without the presence of the fungus. Petri dishes inoculated was used for the tests after 1 week of inoculation in order to permits fungal colony establishments and expansion. Petri dishes contaminating during propagation and experiment was excluded from the experiment.

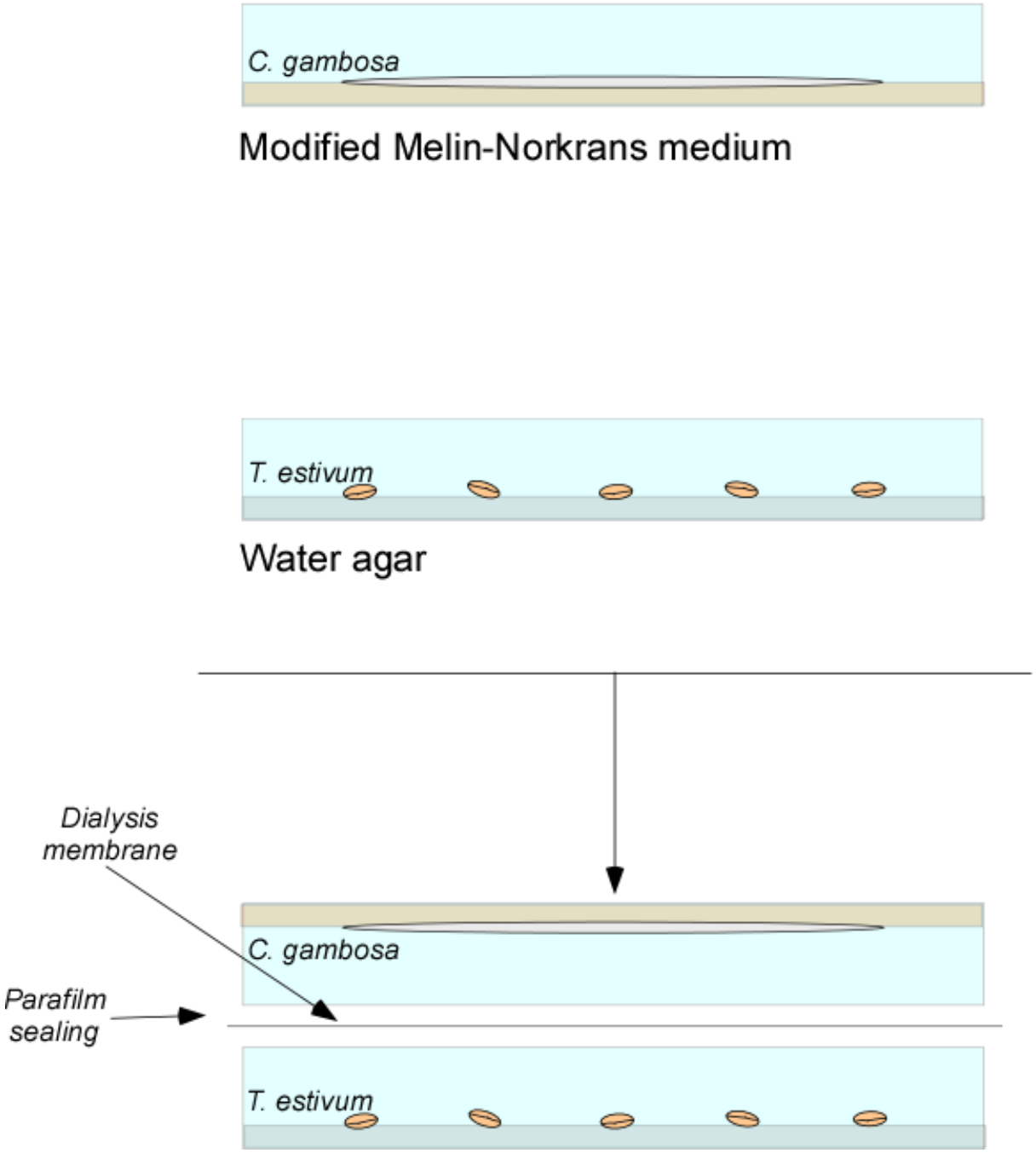

Figure 4.4.1 Overlapped petri dishes used for the experimental tests.

Data collection and Statistical analysis

We perform tests in Friday and data collection in Monday. We measured number of germinated seeds, root length, number of roots, number of shoots and shoot length. In detail we carefully opened petri dishes containing *T. estivum* seeds in sterile hood. Following, seeds were placed on squared paper sheet divided by treatment and petri dishes and photographed. Subsequently data was obtained by image analysis through ImageJ software. For the second experiment, average percentage germination data and root length we calculated the Vigor index according to the formula:

$$VI = [Percentage\ of\ Germination * Average\ Root\ Length]$$

Data was analysed for significative variations between the treatments by MANOVA (multivariate analysis of variance) and specific significative variation assessed according to Duncan *post-hoc* test. Significance threshold fixed for p-values below 0.05.

4.4.3. Results

In the first experiment we observed germination of *T. estivum* in presence and absence of volatiles compounds from the mycelium of the FR forming *C. gambosa.* Overly, is observed an increase for most of the seed parameter measured in presence of the fungus. The general promotive effect of VOCs released by the fungus on the seeds of *T. estivum* is also confirmed by MANOVA. In detail, promotive effect is observed for Number of roots, Average root length, Total root length, Number of shoots and finally Germination that is the only one parameter assuming significant variation within the treatments (*p-values 0.0134*).

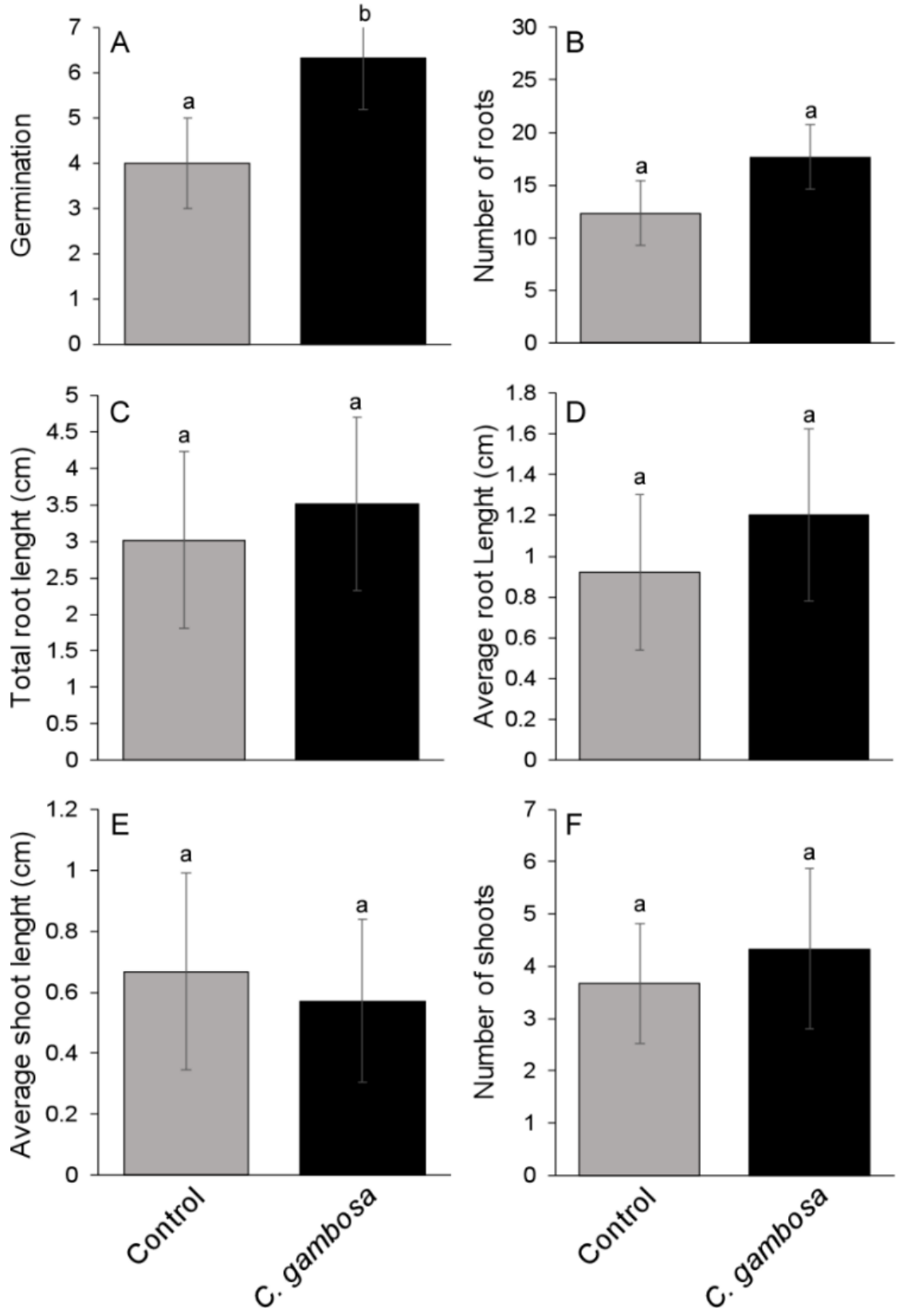

Figure 4.4.2 Bar plots showing the results of first experimental section of seed germination and growth of *T. estivum* in presence and absence of *C. gambosa.* Specific significant variation is showed by different letters between treatments. Significance assessed by Duncan tests for p-value below 0.05

Table 4.4.1 Result of Multivariate analysis of variance (MANOVA) and univariate analysis of variance (ANOVA) of first experiemnt. Significant values for p-value below 0.05 are showed in bold.

		Value	F	Effect	Error	p
Multivariate	Intercept	0.226809	19.88576	6	35	***0.000000***
	Treatment	0.685434	2.67708	6	35	***0.030341***
		SS	Degr. Of freedom	MS	F	p
Univariate	Germination	1.16667	1	1.16667	6.7123	***0.013298***
	Number of roots	6.0952	1	6.0952	3.1644	0.082861
	Total root lenght	2.5279	1	2.5279	0.22821	0.635451
	Number of shoots	0.09524	1	0.09524	0.37383	0.544382
	Shoot lenght	0.09400	1	0.09400	0.17844	0.674982
	Average root lenght	0.84596	1	0.84596	0.85264	0.361342

In the second experiment we applied different inoculum points in order to confirm the effect observed in the first experiment and in order to observe whether the promotive effect from the *C. gambosa* mycelium increase with its own biomass. At the beginning of the experiment we tested a total of 20 petri dishes. At the end, 19 petri dishes were included in the measurements because of a contamination of one belonging to the 1 plug group. Comparison of seeds measurements growing in petri dishes interacting without and with one, two and four colony of *C. gambosa* showed a gradual increase in seeds germination and metrics in cooccurrence with the increasing biomass of the fungus. In detail, the promotive behavior is significatively verified for all the metrics of the 4 plugs treatments when compared with the other treatments. Only exception is made for 2 plugs treatment enhancing germination in a significant way compared to control and 1 plugs treatment.

Table 4.4.2 Result of Multivariate analysis of variance (MANOVA) and univariate analysis of variance (ANOVA) of second experiment. Significant values for p-value below 0.05 are showed in bold.

		Value	F	Effect	Error	p
Multivariate	Intercept	0.372663	33.10661	6	118.0000	***0.000000***
	Treatment	0.517368	4.87194	18	334.2397	***0.000000***
		SS	Degr. Of freedom	MS	F	p
Univariate	Germination	5.51278	3	1.83759	8.8640	***0.000022***
	Number of roots	61.4959	3	20.4986	10.7621	***0.000002***
	Total root lenght	268.075	3	89.3583	8.10763	***0.000055***
	Number of shoots	7.66917	3	2.55639	15.38947	***0.000000***
	Shoot lenght	29.02416	3	9.67472	13.48867	***0.000000***
	Average root lenght	18.3728	3	6.1243	5.87234	***0.000864***

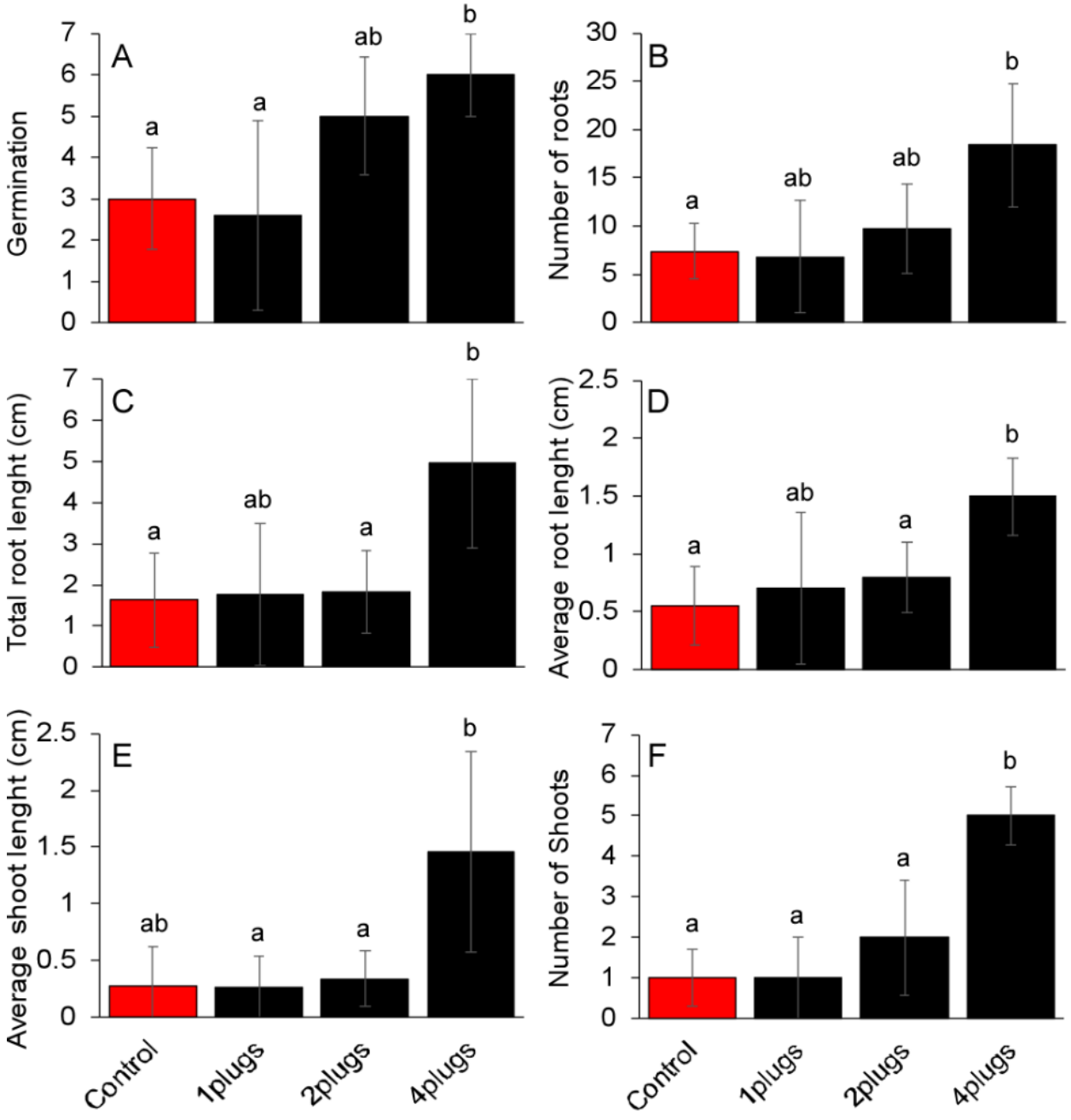

Figure 4.4.3 Bar plots showing the results of second experimental section of seed germination and growth of *T.estivum* in absence and increasing gradient of presence of *C. gambosa.* Specific significant variations are showed by different letters between treatments. Significance assessed by Duncan tests for p-value below 0.05

4.4.4. Discussion

In the present experiment we demonstrate that the FR forming fungus *C. gambosa* stimulate germination of *T. estivum* seeds. Nowadays, studies on FRs describes the FRs as ecosystem engineer, although the mechanisms in which they act is still not fully understood (Bonanomi *et al.*, 2012; Yang *et al.*, 2019). This because of the lack in the identification of the interactions of the fungus with the extant plant community into the affected ecosystems. A plethora of studies was accumulated in the recent bibliography with several

hypothesis concerning the description of how the FR fungus trigger changes in plant community (Edwards, 1988; Blenis *et al.*, 2004; Gramss *et al.*, 2005). Surprisingly, our study showed a growth promoting effect from VOCs released by colonies of the FR-fungus. These results are in discordance with those illustrating a detrimental effect of the FR fungi by release of toxic compounds as such as volatile cyanohydrins(Blenis *et al.*, 2004). These results bring us to think that, among the four main hypotheses formulated to explain the detrimental effect characterizing the Type 1 FRs, release of toxic compounds could not be take in consideration. However further experimental evidence and tests are needed in order to disentangle whether the claim of toxic compounds mechanisms could be excluded. For example, a direct application of a water extract from the secondary metabolites produced by mycelium are needed to obtain major and useful information. Additionally, we tested the effect in only one FR fungus species, that in light of the extreme variability and immense range of volatiles produced by each FR species, suggest us that our approach is reductive (Morath *et al.*, 2012).

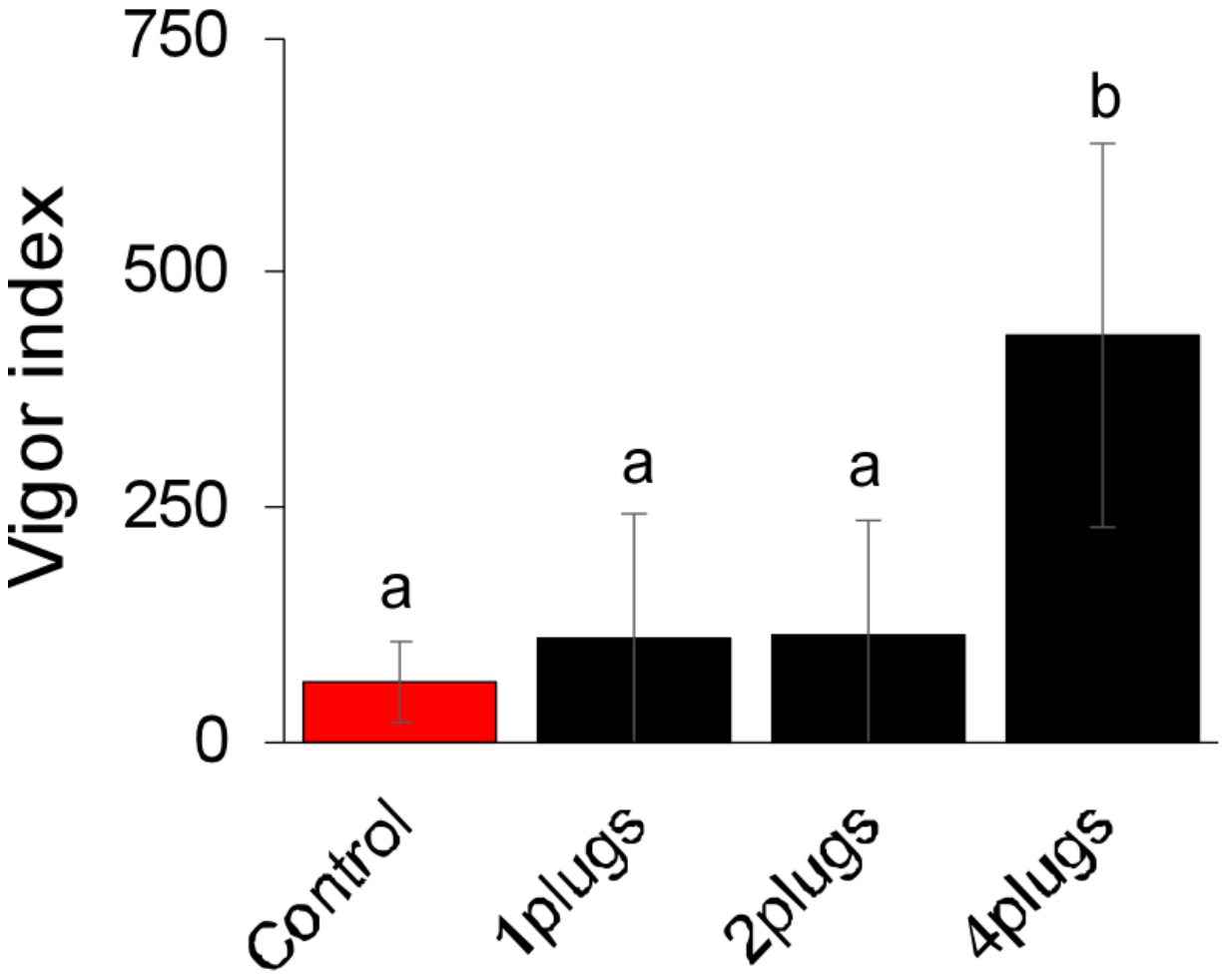

Figure 4.4.4 Bar plots showing the results of second experimental section for Vigor index of *T. estivum* seeds in absence and increasing gradient of presence of *C. gambosa.* Specific significant variations are showed by different letters between treatments. Significance assessed by Duncan tests for p-value below 0.05

However, our results are in accordance with test conducted on the FR fungus *L. sordida,* that was assessed has a stimulating effect on rice (Choi *et al.*, 2019). Unfortunately, a full comparison between the present results and those obtained with *L. sordida* is not possible, first because test of the effect of *L. sordida* was conducted in soil and second because researcher used extract from the fungus and not the active source of chemicals (Choi, JH *et al.*, 2010; Choi *et al.*, 2014). Notwithstanding, is probable that the effect observed, first with increased germination, and later with a growth promoting effect is derived by patterns similar to those observed in *L. sordida.* Indeed, is probable that VOCs released in the environments trigger hormonal response in plants organisms increasing their ability to incorporate nitrogen and other nutrient, upregulating aquaporines synthesis, and increasing the ability to detoxify internal environment from xenobiotics and defense against pathogens (Choi *et al.*, 2014; Kawagishi, 2018; Kawagishi, 2019). Interestingly, the experiments that brings

to the discovery of the "Fairy Chemicals" were aimed to describe a growth promoting effect instead of a germination. Oppositely our works is aimed to describe an effect on germination that we know follow a completely different pattern. Indeed, germination is regulated by consumption of internal level of Abscisic Acid (ABA) (Williamson & Quatrano, 1988; Lane, 1991; Finkelstein & Gibson, 2002). Consumption of the stocks of ABA promote a cascade signaling that terminate in the break of dormancy in the seeds. Nowadays there are no evidence of these effect from VOCs produced by basidiomycetes, but exceptionally, the effect was described for some bacteria strains able to produce volatile Indole that is included in auxins metabolic pathway, or other molecules with uncertain effect like 3-hydroxy-2-butanone, 2,3-butanediol, 2-nonanone, 2-undecanone, 2-tridecanone and 2-pentadecanone (Ann *et al.*, 2013; Yu & Lee, 2013; Bailly *et al.*, 2014), or was described as a negative effect from voc released by Ascomycetes (Splivallo *et al.*, 2007)

More probably, the mechanisms observed in the present study could explain patter of formation of Type 2 FRs or the formation of the flourishing vegetation in the Type 1 FRs. In matter of fact, the study of the detrimental effect of Type 1 FRs attracted the attention of the ecologists, because of their dramatic capability to changes soil community structures. Inversely few attentions were posed in the mechanisms behind type 2 FRs. Is quite probable that both FR typologies has the ability to promote growth and germination of plants by release of VOCs. Inversely, the formation of the area with dead and poor vegetation could be ascribed to the capability of the FR fungus to induce hydrophobicity. As it is that induced hydrophobicity is dependent by the biomass of the fungus (because of the release of hydrophobines), the first observation from Shantz and Piezemel (1917) was outstandingly useful. Comparing colonization of mycelium in soil sod between Type 1 and Type 2 FRs was observed that fungal body occupation was less present in the last. As a general observation this could explain the mismatch in classification for fungi as *M. oreades* or *A. campestris*, that are classified both as Type 1 or Type 2, because of different forms of colonization of soils that could be dependent by seasonality or fungal genotype.

4.4.5. Conclusion

The results from this preliminary microbiological test suggest that the mechanisms promoting detrimental effect of the Type 1 FR should be addressed to instauration of hydrophobicity in soil instead to the production of phytotoxic compounds like cyanohydrins. The present work furnishes an advancement in understanding pattern of species richness in terrestrial ecosystems. Indeed, in light of the importance of FRs as ecosystem engineer the understanding of the specific interaction produced by the fungus is crucial for the management and conservation of important ecosystems as grassland or could provide useful information for a future application in agricultural field. However, more extensive studies are required in order to obtain full explanation of the process.

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5. Litter decomposition and water relations produce negative feedbacks in aquatic plants

Overview

Leaf litter decomposition release nutrients that support ecosystem productivity, but may also release toxic compound with harmful effect on plant root. Most of previous studies were carried out in laboratory, with no available experiment in stream ecosystem. The aims of this work were to assess the inhibitory effect of leaf litter and purified self and heterologous DNA on aquatic root of *Alnus glutinosa*, a common riparian woody species. To this purpose, we conceived and developed a novel method using plastic tube fixed to a single root and filled with leaf litter or pure chemical compounds. In a first experiment we tested the effect different eight litter types, both fresh and decomposed for six months. Litter chemistry was characterized by ^{13}C-CPMAS NMR, extractable C, cellulose, lignin, and N content. Moreover, the effect of water flux was assessed by creating closed and open systems where litter leachate were either continuously removed or left in contact with tree roots. A second experiment was done challenging *A. glutionsa* root with purified self- and heterologous DNA. We found that leaf litter has an age-dependent effect on *A. glutinosa* root, producing a major inhibitory effect when fresh and in the closed system. After decomposition, however, litter toxicity decreased with the inhibitory effect positively correlated with extractable C and negatively with lignin and lignin/N ratio. In the second experiment self DNA, but not heterologous one, caused an acute toxic effect in the closed system. In the open system, instead, the harmful effect of self DNA was radically reduced. We provided a method that will allow to study the impact of leaf litter, as well as of pure chemical compounds, in field conditions in aquatic ecosystems.

5.1. Direct effect of phytotoxicity of differently decomposed litter on root of riparian species

5.1.1. Introduction

Leaf litter represent a substantial fraction of plant detritus that periodically return to the soil system and undergoes the decomposition pathway (Aerts, 1997a). Plant litter is the main input of organic carbon and nutrients that feed the biogeochemical cycles, but leaf litter also play an important role in shaping plant community composition and structure (Facelli & Pickett, 1991). The presence of a litter layers over grassland soil or a forest floor can either promote or inhibit seed germination, seedling establishment (Jensen & Gutekunst, 2003), plant root growth and nutrition (Cuevas & Medina, 1988; Conn & Dighton, 2000), protection from desiccation (Loydi *et al.*, 2013), release of mineral nutrients during decomposition to support plant nutrition (Hobbie, 2015), and the support of a beneficial microbiota in the soil systems (Hättenschwiler *et al.*, 2005) are all positive effects associated with litter presence. On the other hands, inhibitory effects of leaf litter has been reported by a number of studies (Xiong & Nilsson, 1999) and are caused by because litter act as a physical barrier (Scarpa & Valio, 2008) or, more commonly, by a chemical interference effect (Rice, 2012). In this latter case, plant inhibition is the results of a combination of nutrient starvation and chemical toxicity due to allelopathic compounds. Nutrient starvation mainly involve nitrogen (N) and is caused by microbial competition when decomposing organic matter had a C/N ratio above the threshold values of ~30 (Hodge *et al.*, 2000). In presence of N poor plant leaf litter, large root or wood debris, microbe outcompete plants for mineral N uptake physically moving this resources into the organic matter (Lummer *et al.*, 2012) and, as a consequence, cause a deprivation of mineral N in the surrounding soil that may impair plant growth. The intensity and duration of N immobilization depended on the amount and C/N ratio of the considered litter, and may last from few week as in case of leaf litter to several years when large amount of wood is involved as occur after hurricanes or large disturbance (Zimmerman *et al.*, 1995). Direct litter phytotoxicity is also widespread, with three studies that reported an inhibitory effect for with 21 (Lopez-Iglesias *et al.*, 2014), 64 (Bonanomi *et al.*, 2011) and 65 (Meiners, 2014) litter types in temperature and Mediterranean ecosystems. A wide array of allelopathic compounds has been isolated and identified, especially in water leachate of fresh litter and during the early phases of decomposition (Rice, 2012). The most common phytotoxic compounds in litter include short-chain organic acids as propionic and butyric acids (Armstrong & Armstrong, 1999), tannins (Kraus *et al.*, 2003), and low molecular weight phenols (Li *et al.*, 2010). In this regard, previous studies also clarified that litter phytotoxicity is largely affected by plant functional type and the stage of decomposition. In detail, leaf litter is usually more phytotoxic than root debris and among plant functional type tissues of nitrogen-fixing species are, in average, more inhibitory than forbs, woody species and grasses (Bonanomi *et al.*, 2017b). Moreover, it is also well established that a rapid transformation and degradation of most labile allelochemicals compound into non-toxic molecules causes a rapid, usually in the time-frame of weeks or few months, of the litter phytotoxic effect (Chou & Patrick, 1976; Bonanomi *et al.*, 2006a; Dorrepaal, 2007). A notable exception to this general patter is the inhibitory effect caused by litter released by conspecific. In this cases plant debris have a species-specific autotoxic effect (Singh, H *et al.*, 1999), with inhibition that is long-lasting, up to several years (Cesarano *et al.*, 2017b). Such species-specific and persistent autotoxicity has been associated to the release

and accumulation of extracellular DNA (exDNA) in the litter layer and underlying soil during decomposition (Mazzoleni *et al.*, 2015a). Noteworthy, a series of laboratory experiments highlighted that exDNA toxicity is depended on fragment size, with the major inhibition observed for fragments that range between 50 and 2000 bp. However, all published studies up to now has been carried out in laboratory conditions (Mazzoleni *et al.*, 2015c; Barbero *et al.*, 2016; Duran-Flores & Heil, 2018), with no yet evidence of exDNA toxicity in field conditions.

The impact of leaf litter on plant community depends on the amount, spatial distribution and chemical quality of plant debris. In other words, the expected impact of litter phytotoxicity and autotoxicity may depend on their physical distribution, cycling pathway, and decay rate in different ecosystems. For instance, in fire prone plant communities leaf litter is periodically removed and transformed in in a highly heterogeneous mixture composed of charred substrates and mineral ash (González-Pérez *et al.*, 2004). In riparian ecosystem, instead, leaf litter is periodically removed by water flux and spatially re-arrangement, creating a mosaic of bare areas and zone with notable accumulation of litter, i.e. leaf litter pack (Kominoski *et al.*, 2007; Peralta-Maraver *et al.*, 2011). In ecosystems with regular water flush, (i.e. wetlands and marshes, floating vegetation, riparian forests and mangrove stands, seaweed and seagrasses as well as kelp forests), the periodical removal of litter combined with the dilution of the dissolved organic fraction leached from litter may drastically reduce the potential impact of litter on root growth (Mazzoleni *et al.*, 2010). On the contrary, a strong impact of leaf litter could be expected in proximity of local litter accumulation as occur in under leaf litter packs observed in stream.

In this study, we conceived and developed a novel method to test the effect of plant litter, as well as of pure chemical compounds, on root of tree species in field condition in riparian ecosystems. Most of previous studies about litter allelopathic effect are carried out in laboratory conditions using very sensitive, short-lived species (Macías *et al.*, 2000; Weston, 2000), leaving doubt about the real impact of such compounds in field conditions. Here, the novel method based on the use of plastic tube fixed to a single root and filled with leaf litter or pure chemical compounds, was applied on *Alnus glutinosa.* We select this species because this tree, common in riparian area, develop aquatic roots that, thanks to their coarse structure and physical resistance, are suitable for a manipulative experiment. In this study, we firstly tested the effect different litter types, both fresh and decomposed for six months. Litter chemistry was characterized by ^{13}C-cross-polarization magic angle spinning nuclear magnetic resonance (^{13}C-CPMAS NMR) spectroscopy (Kögel-Knabner, 2002) as well for proximate cellulose, lignin, and C and N content. Moreover, the effect of water flux was explored by creating closed and open systems where litter leachate was either continuously removed or left in contact with tree roots. Finally, an experiment was carried out to assess the impact of purified self- and heterologous DNA on *A. glutionsa* root. Specific hypotheses tested in this study were:

i. litter had higher inhibitory effects when fresh than after six months of decomposition;

ii. litter inhibitory effect vary among species and such variability can be explained by chemical descriptors;

iii. self DNA exert inhibitory effect on root of conspecific with no effect expected for heterologous DNA;

iv. litter and self DNA inhibitory effect would be stronger in closed than in open systems.

5.1.2. Material and methods

Study site description

The experimental activities were carried out in a stream tributary of the Alento river in located in Cicerale, southern Italy (elevation of 180 m a.s.l., 40°19'49.17''N; 15°07'28.28''E). The riparian forest along the stream is dominated by the native trees *A. glutinosa*, *Populus nigra* L., *Salix alba* L., *Quercus ilex* L. and the invasive, nitrogen-fixing tree *Robinia pseudoacacia* L.. The dominated vegetation layer is composed of vines likes *Hedera helix* L., *Clematis vitalba* L., *Rubus ulmifolius* Schott and *Smilax aspera* L., with a dense herbaceous layer with grasses (i.e. *Dactylis glomerata* L., *Brachypodium rupestre* Host, *Festuca drymeja* Mert.), many forbs and short-lived nitrogen fixing species (details on site vegetation are reported in (Incerti *et al.*, 2018). The study site has a Mediterranean climate with a mild and wet winters and a relatively hot and dry summers. Mean annual temperature is 14.8 °C, with a mean monthly temperature that range between 24.4°C (August) and 6.8°C (January). The mean annual rainfall is high (1,328 mm), distributed mainly in winter, autumn, and spring with a notable rainfall deficit in summer.

Table 5.1.1. Physical and chemical parameters of the soil of the study site.

Parameters	**Riparian forest (Cicerale)**
Sand (g kg^{-1})	622 ± 97
Silt (g kg^{-1})	225 ± 458
Clay (g kg^{-1})	172 ± 32
pH	6.89 ± 0.22
EC (dS m^{-1})	0.10 ± 0.02
Organic C (g kg^{-1})	28.11 ± 3.2
Total N (g kg^{-1})	3.11 ± 0.32
Limestone (g kg^{-1})	2.35 ± 1.32
P_2O_5 (mg kg^{-1})	20.31 ± 2.21
CEC (meq 100 g^{-1})	27.31 ± 5.7
K^+ (meq 100 g^{-1})	2.16 ± 0.53
Mg^{2+} (meq 100 g^{-1})	3.22 ± 0.37
Ca^{2+} (meq 100 g^{-1})	43.38 ± 3.91
Na^+ (meq 100 g^{-1})	0.22 ± 0.72
Cu (mg kg^{-1})	0.98 ± 0.13
Zn (mg kg^{-1})	1.19 ± 0.12
Mn (mg kg^{-1})	6.15 ± 1.15

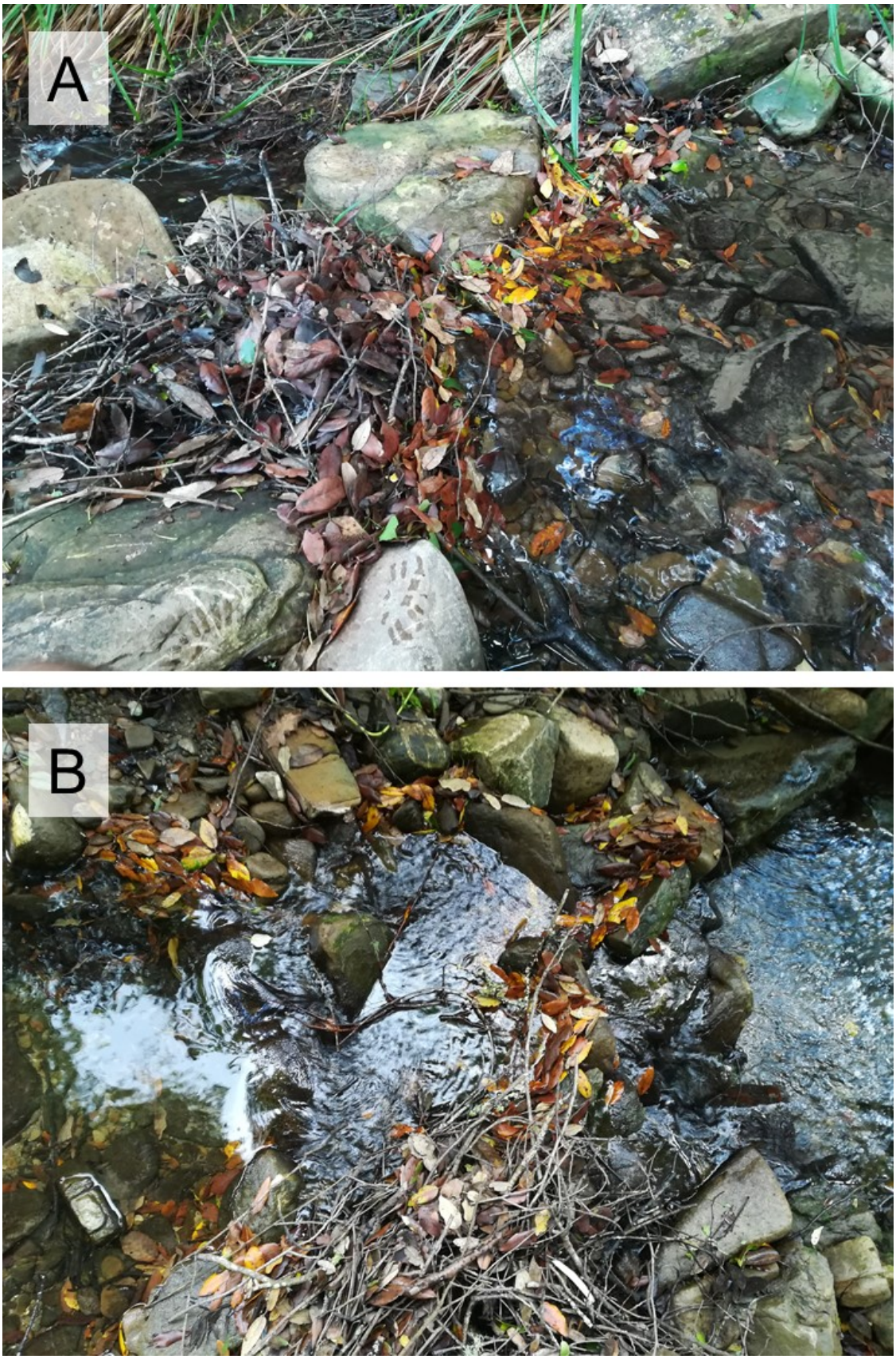

Figure 5.1.1. A, B: selected pictures of the study site in June 2018 showing the formation of leaf litter and wood debris packs, note also the amount of standing litter in ponds. An even higher litter concentration is commonly observed in July and August because of the reduced water flux of the stream.

Species selection, decomposition experiment and chemical analyses

Litter of four species belonging to different functional groups and coexisting at the study site were selected i.e. *H. helix* (evergreen vine), *F. drymeja* (perennial grass), *A. glutinosa* (deciduous, nitrogen fixing tree), and *P. nigra* (deciduous tree). The four litter types represent a wide range of litter chemistry in terms of nitrogen (N) and lignin and C/N and lignin/N ratios. For trees, litter was collected by placing nets under the selected plants in the study site (number of plants = 5, individuals were randomly selected). For *H. helix* and *F. drymeja* litter freshly abscised leaves were collected manually. Collected litter was transferred to the laboratory, air dried at room temperature (ventilated chamber) and thereafter stored in paper bags.

The decomposition experiment was carried out in filed condition using the litterbag method (Berg & McClaugherty, 2014). Briefly, large (30 x 30 cm) terylene litterbags (mesh size 1.5 mm) were filled with 10 g of litter and placed over the soil in the riparian ecosystem. The characteristics of the soil are reported in Table 5.1.1. At the planned harvesting date (180 days since the start of the experiment), litterbags were collected, air dried in a ventilated chamber (35°C until constant weight was reached). The experiment produced 8 organic materials (4 species either undecomposed or decomposed for 180 days) indicate, thereafter, as fresh and aged litter.

Fresh and aged litter were analysed for total organic carbon (C) and N using an elemental analyser NA 1500 (Carlo Erba Strumentazione, Milan, Italy). The content of acid-detergent hydrolysable fraction (hereafter indicated as extractive C), cellulose, and proximate lignin were quantified with the method of (Gessner, 2005). Briefly, cellulose was quantified as hydrolysable fraction after treatment with sulphuric acid (weight loss due to 72% H_2SO_4), and lignin as the unhydrolyzable fraction and quantified after loss upon ignition of the fraction remaining after the H_2SO_4 treatment. The metabolome of the 8 organic materials were also defined by ^{13}C-CPMAS NMR obtained in solid state. Analyses were carried out under the same experimental conditions in order to allow a quantitative comparison among obtained spectra. The analyses were carried out using a spectrometer Bruker AV-300 equipped with a 4 mm wide-bore MAS probe (details in Bonanomi et al. 2017). The spectral regions selected for analysis and corresponding to different C-types were identified following (Almendros *et al.*, 2003), (Kögel-Knabner, 2002) and Bonanomi et al. (2017). Briefly, the spectra was separated in the following seven regions: 0-45 ppm = alkyl C; 46-60 ppm = methoxyl and N-alkyl C; 61-90 ppm = O-alkyl C; 91-110 ppm = di-O-alkyl C; 111-140 ppm = H- and C- substituted aromatic C; 141-160 ppm O-substituted aromatic C (phenolic and O-aryl C); and 161-190 ppm carboxyl C.

Experiment 1: leaf litter effect on A. glutinosa root

We selected *A. glutinosa* as target species because during the growing season, from May to November, develop aquatic roots. These root start to develop in May and after ~3 weeks of elongation phase reach a length of 10-14 cm, thereafter remain vital without further elongation until November (G. Bonanomi, *personal observations*). Roots has a diameter of ~2-3 mm and are sufficiently resistant to allow a manipulative experiment (Figure 5.1.2). At the study site also *S. alba* produce a large number of aquatic roots but these roots appear physically fragile and don't allow even a minimal manipulation and, for this reason, this species was excluded from the experiment. Finally, the other tree species present along the stream (*P. nigra*, *R. pseudoacacia*, *Q. ilex*) don't produce aquatic roots.

The first bioassay, referred to as the 'root-litter interaction' experiment, was conducted in field condition with the aim of assessing the short-term impact of leaf litter on vitality of tree root. To this purpose we develop a new method based on the use of plastic tube filled with different litter type (Figure 5.1.2). We used plastic tubes 5 cm long, a diameter of 0.8 cm and a total volume capacity of 4.5 ml. Tube were leaf intact to create the "closed" system while 10, 1.5 mm holes were made in the tube to create the "open" system that allow the free circulation of stream water. In the experiment, a tube was applied to a single *A. glutinosa* root. The root was introduced in the tube and fixed using a portion of hydrophobic cotton. The tube was filled with 0.1 g of dry litter and 4 ml of water, equivalent to an application rate of 25 g L^{-1}. Experimental values of litter application rate is consistent with the levels of litterfall observed in natural ecosystems (Abelho, 2001) and with previous studies carried out in laboratory conditions e.g. (Bonanomi *et al.*, 2011). Moreover, at the study site periodically leaf pack are formed in the stream, reaching high litter concentration in water especially in summer when the water flux is reduced (Figure 5.1.1). This set-up was used because allow the contact between litter, litter leachate and root in closed system and between litter and root in the open one, mimicking the real ecological conditions observed at the study site.

Overall, the experimental design includes 8 litter types (4 species, fresh and aged), the closed and open system, with a single litter application rate (25 g L^{-1}). Control tubes, both for closed and open system, were filled only with water. Each treatment was replicated ten times, then overall 180 roots were selected (8 litter types, 2 systems, 10 replicates plus 20 for the controls). Five adult *A. glutinosa* trees rooted within 1 m from the stream were selected, having height ranging between 8 and 12 m and d.b.h. from 12 to 18 cm. For each tree we selected 36 roots and all treatments were applied to each tree, i.e. 36 tubes for tree that include all treatments with two replications. The experiment was run in June 2018 and the experiment latest 72 hours and at the end the roots were excited and processed. The short duration of the experiment was decided because our aim was to assess the acute effect of litter contact with roots. Immediately after excision, high resolution pictures of roots were taken by a Nikon D5. Thereafter, roots were conserved in ethanol (90%) for histological analyses.

Concerning data analysis, for each root the lengths portion that were either vital or necrotic were measured by image analyser software (Image J). Histological analyses were carried out according to (De Micco & Aronne, 2010) in order to obtain information on the modification of the internal structures of the roots following litter application. Coherently with experimental design, each replica belonging to the same treatment were included in a single slide and analysed by microscopy. At 40 x magnification in normal and fluorescence conditions. Anatomical features of water roots of *A. glutinosa* were described in control treatments, meanwhile, damages were quantified by different parameters as such as, number of cellules and thickness of epidermis layers, regular shapes and presence or absence of fragmented and/or dysmorphic cellules.

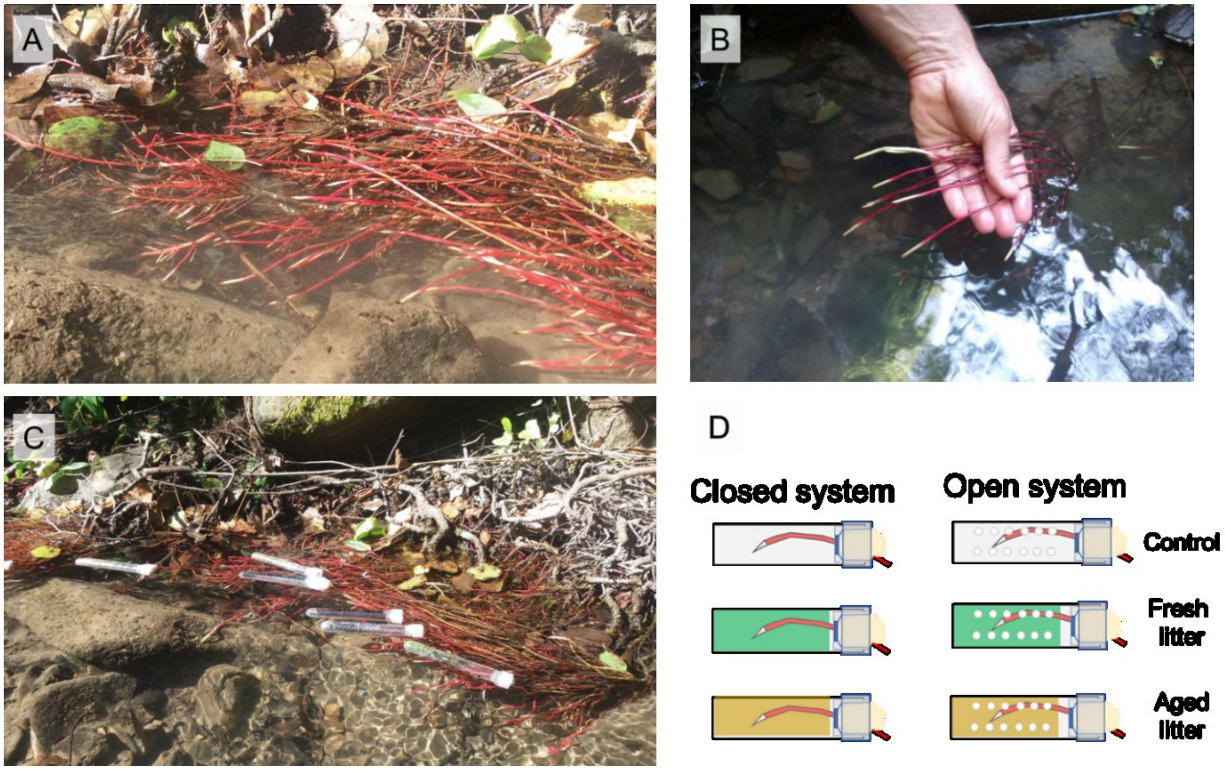

Figure 5.1.2 Selected images of the study site and the experimental system. A, B: aquatic roots of Alnus glutinosa in June 2018, hand for scale; C: tubes applied to Alnus glutinosa tree to challenges roots with different leaf litter types; D: schematic representation of the factors included in the first experiment showing the closed (intact tubes) and open (pierced tubes with holes to allow water circulation) systems. Tubes either were filled with water (controls), fresh (green) or decomposed (brown) litter for 180 days.

Experiment 2: self- and heterologous DNA effect on A. glutinosa root

The second experiment was conducted in field condition with the aim of assessing the short-term impact of self and heterologous DNA on vitality of tree root. To this purpose we use the same method applied for leaf litter but tubes was filled with purified DNA. The experimental design includes 2 DNA types, i.e. self-DNA extracted from *A. glutinosa* leaf and heterologous DNA extracted from *F. drymeja* leaf. DNA was extracted CTAB methods. DNA was fragments by sonication and applied in the tubes at concentration of 50 ppm. Selected DNA concentration is within the range used by previous laboratory studies (Mazzoleni *et al.* 2015a and b). The experiment was carried out using the closed and open system, with a single litter application rate (50 ppm), with ten replicates. Control tubes, both for closed and open system, were filled only with water. Overall, 60 roots were selected (2 DNA types, 2 systems, 10 replicates plus 20 for the controls) (Figure 5.1.3). Four adult *A. glutinosa* trees rooted within 1 m from the stream were selected, having height ranging between 8 and 12 m and d.b.h. from 12 to 18 cm. For each tree we selected 15 roots and all treatments were applied to each tree. The experiment was run in June 2019 and, also in this case, the experiment latest 72 hours and at the end the roots were excited and processed as previously described.

Figure 5.1.3. A: picture of the study site in June 2019 showing a typical pond in the stream with a large *Alnus glutinosa* tree (right, upper corner). B: tubes applied to *Alnus glutinosa* tree to challenges roots with heterologous and self-DNA.

Data analyses

We tested effective variation in root damage by Univariate Factorial ANOVA. We compared variation of root damages depending to Litter type (LT), Litter decomposition stages (LD), open/closed system (SY) and interactive effect between the treatments. Result of ANOVA were visualized for significant variation by *post-hoc* Duncan test. Significative variation for p-values below 0.05. subsequently, values of root damage were crossed with chemical characteristics of litters by calculation of Pearson correlation coefficients. Statistical were performed by means of Statistica 10 software.

5.1.3. Results

Leaf litter chemistry

Litter chemistry varied among species and with decomposition time (Table 5.1.2). Among undecomposed materials, *F. drymeja* had the highest lignin/N ratio while *P. nigra* recorded the lowest N content. The nitrogen fixing *A. glutinosa* and *H. helix* showed high N content coupled with the low C/N and lignin/N ratio. *A. glutinosa* had the highest concentration of extractable C, *F. drymeja* the lowest with intermediate values for *H. helix* and *P. nigra*. As expected, C/N ratio, extractable C and cellulose content significantly decreased with litter age for all species. On the contrary, lignin content and lignin/N ratio generally increased with litter age, with higher rates in the case of *H. helix*. During decomposition N concentration increased for all species with only exception for *H. helix*.

^{13}C NMR spectra highlight consistent changes of litter chemistry during the 180 days of decomposition. First, the relative abundance of O-alkyl-C and di-O-alkyl-C fractions, which largely correspond to sugars and polysaccharides, decreases during decomposition for all species, with the most notable reduction for *H. helix*. On the contrary, the relative content of alkyl-C fraction, characteristic of lipid as waxes, cutins and microbial spoilage, increased for all species, especially for *H. helix, F. drymeja* and *P. nigra*. The relative abundance of aromatic C showed minor changes, while the carboxylic C fraction showing a minor increase for *F. drymeja* and *P. nigra*.

Table 5.1.2. Leaf litter chemical traits of the four tested plant species at two decomposition ages (0 and 180 days) assessed by elemental, proximate analyses and ^{13}C CPMS NMR.

	Elemental and proximate parameters						**^{13}C-CPMAS NMR-derived parameters**						
	Extractable C (%)	Cellulose (%)	Lignin (%)	N (%)	C/N	Lignin/N	Carboxylic C – 161-190 ppm	O-substituted aromatic C – 141-160 ppm	H-C-substituted aromatic C – 111-140 ppm	di-*O*-alkyl C – 91-110 ppm	*O*-alkyl C – 61-90 ppm	Methoxyl C – 46-60 ppm	Alkyl C - 0-45 ppm
Alnus 0 days	64,.30	18.7	13.12	2.07	21.73	4.53	8.3	3.2	11.9	8.1	35.1	8.4	24.4
Alnus 180 days	46.64	21.43	23..71	2.91	15.12	6.42	8.5	3.9	13.9	6.7	27.4	10.2	28.2
Festuca 0 days	53.01	26.96	16.15	1.70	26.52	9.50	4.4	4.2	12.3	17.5	61.8	0.3	0.1
Festuca 180 days	49.12	13.52	28.56	1.87	12.86	15.31	7.0	4.1	12.2	10.1	37.6	10.3	21.3
Hedera 0 days	70.46	23.6	5.7	2.0	22.9	2.89	7.2	2.7	5.9	8.9	43.2	6.8	25.3
Hedera 180 days	60.52	10.8	26.6	1.82	16.9	14.55	7.1	2.8	8.0	6.1	21.5	7.3	49.3
Populus 0 days	61.46	22.49	12.51	1.53	25.01	8.20	7.1	5.5	11.6	12.7	43.1	6.3	16.0
Populus 180 days	47.80	11.50	31.22	2.24	12.03	13.94	8.1	4.0	11.3	8.2	30.2	11.4	29.7

Leaf litter effect on A. glutinosa root

The effect of litter on *A. glutinosa* roots was significantly affected by litter type, decomposition time and type of systems i.e. open *vs.* closed (Table 5.1.3). The application of undecomposed plant litter showed a higher root damage compared to decomposed materials (Figure 5.1.4a). Moreover, the root damage was drastically lower in the open system compared with closed one, for both fresh and decomposed litter (Figure 5.1.4b). Overall, all undecomposed litter caused a significant root damage in the closed system, but the effect varied according to litter type, being highest for *H. helix*, lowest for *F. drymeja* and intermediate for *A. glutinosa* and *P. nigra* (Figure 5.1.4c). When decomposed, and in the closed system, *P. nigra* caused the highest damage, with no difference among the other three species. In the open system, instead, decomposed litter caused very low root damage with *A. glutinosa* litter that caused the highest damage.

By histological point of view is confirmed the damages reported for the visual assessment. Higher internal damage is reported in closed system for roots in contact with *Alnus* litter with few damages on total section surface reported for *Hedera* and *Populus* litter particularly in undecomposed state (Table 5.1.4). Inversely, in opened system higher level of internal damage is reported for undecomposed *Hedera* and decomposed *Alnus*. With increase of damage is observed the decrease of number of epidermis cellules and epidermis

thickness. The pattern is more accentuated in *Alnus* litter that is followed by *Hedera*, *Populus* and *Festuca* litters.

Table 5.1.3 results of Univariate Factorial ANOVA of variation of root damages depending to Litter type (LT), Litter decomposition stages (LD), open/closed system (SY) and interactive effect between the treatments. In bold significant p-values below 0.05

	SS	Degree of Freedom	MS	F	*p*
Intercept	32292.19	1	32292.19	**84.12619**	***0.000000***
Litter type (LT)	3747.40	3	1249.13	**3.25418**	***0.034342***
Litter decomposition (LD)	6650.52	1	6650.52	**17.32564**	***0.000221***
System (SY)	3588.02	1	3588.02	**9.34735**	***0.004485***
LT x LD	3089.06	3	1029.69	2.68250	*0.063286*
LT x SY	8734.90	3	2911.63	**7.58526**	***0.000573***
LD x SY	63.02	1	63.02	0.16418	*0.688034*
LT x LD x SY	9176.56	3	3058.85	**7.96879**	***0.000416***
Error	12283.33	32	383.85		

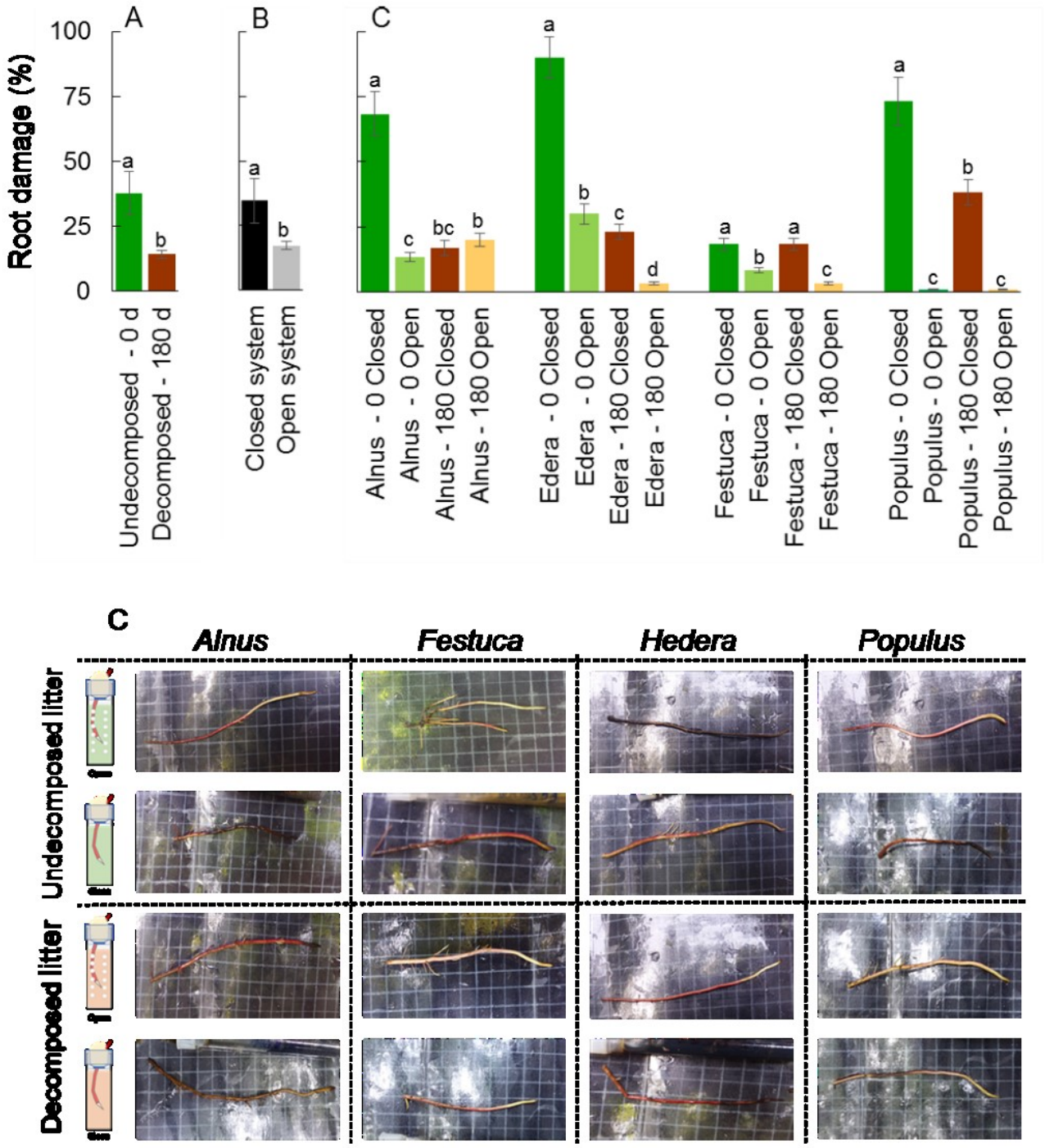

Figure 5.1.4. Effects of 8 litter materials, either undecomposed (green) or after 180 days of decomposition (brown), on *Alnus glutinosa* root in closed and open system. (A) Root damage in undecomposed and decomposed litter, values are species average across. (B) Root damage in the closed and open system, values are average of species and decay rate. (C) Root damage for each litter type in the closed and open system. (D) Selected pictures of A. glutinosa root for each treatment. Within each plot different letters indicate statistically significant differences (Duncan test, $P<0.05$).

Table 5.1.4 Results of histological analysis.

	Epidermis. * thickness	Number of rhizodermal layers	Damaged Area (% respect total)	Internal damage	Irregular shapes	Endoderm fluorescence	Rhizodermis fluorescence
			Open				
Alnus 0 days	61.7	3.0	0.0	-	+	+	+
Alnus 180 days	48.2	2.2	3.7	+	-	+	+
Festuca 0 days	49.0	2.6	0.0	-	-	-	+
Festuca 180 days	65.8	2.9	1.0	-	-	+	+
Hedera 0 days	39.8	2.1	13.5	+	+	+	+
Hedera 180 days	56.7	2.7	0.0	-	-	+	+
Populus 0 days	57.8	3.0	0.0	-	-	+	+
Populus 180 days	51.3	2.3	0.0	-	+	+	+
			Closed				
Alnus 0 days	43.6	2.2	15.8	+	+	-	-
Alnus 180 days	72.2	3.4	1.0	-	+	+	+
Festuca 0 days	78.7	3.5	0.0	-	+	+	+
Festuca 180 days	60.1	2.6	1.9	-	-	-	+
Hedera 0 days	51.6	2.5	2.7	-	-	+	+
Hedera 180 days	70.2	3.5	3.7	-	-	-	+
Populus 0 days	65.5	3.2	4.2	-	+	-	-
Populus 180 days	71.0	3.5	0.0	-	-	+	+

*data expressed as percentage respect control.

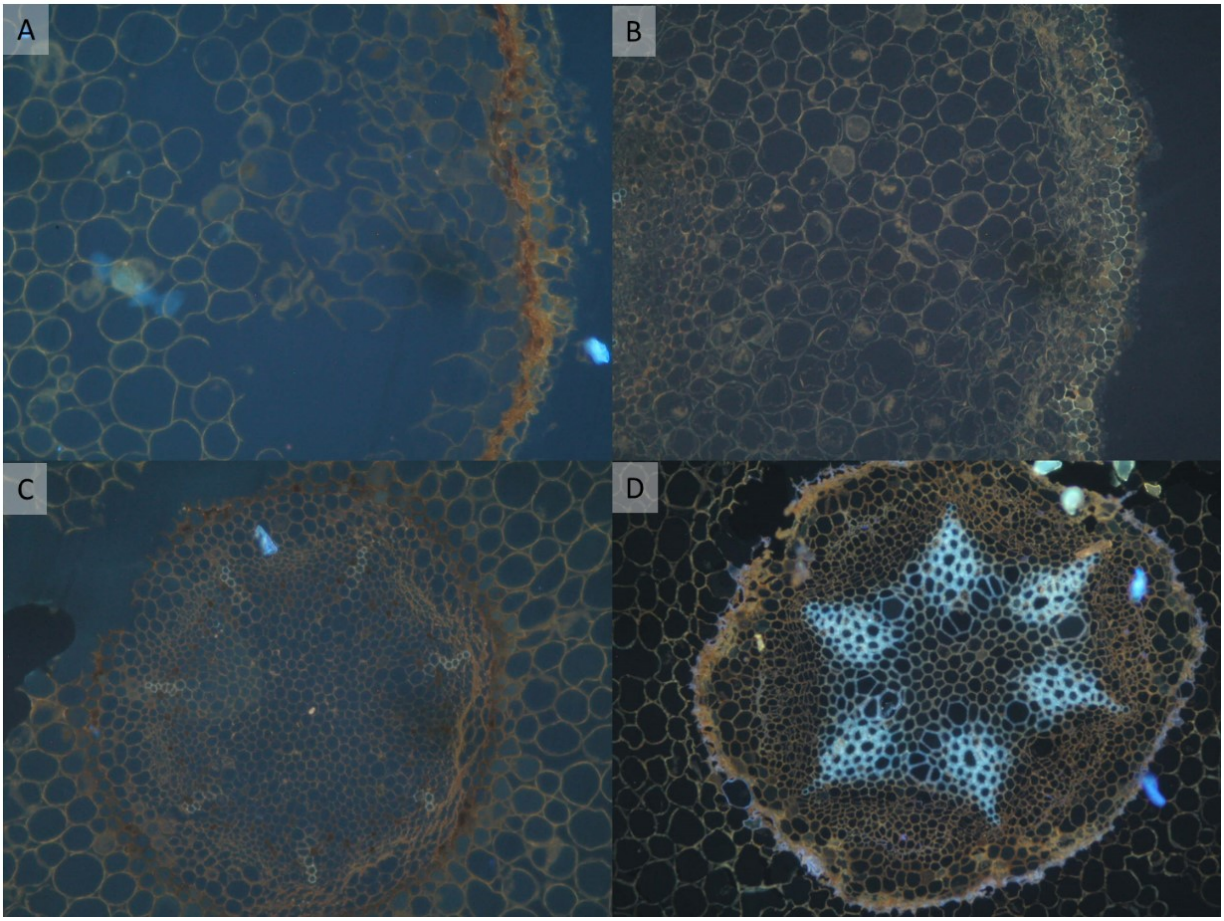

Figure 5.1.5 fluorescence images of root sections 20x magnification (A, B) and 40x magnification (C, D). in panels A, C and B, D detail of Rhizodermis and stele in closed and opened system, respectively.

Leaf litter chemistry and root damage

In the closed system, considering chemical parameters of leaf litter, *A. glutinosa* root damage showed a strong positive correlation with extractable C and C/N ratio (Figure 5.1.6). On the contrary, root damage was negatively correlated with lignin content and lignin/N ratio. N and cellulose concentration was not correlated with root damage in closed system. In the open system we observed a similar correlation pattern with the closed system with only minor variation. In detail, we found that the positive correlation between root damage and extractable C and C/N ratio become weaker, while that with cellulose concentration was significant. Noteworthy, the correlation between root damage and N concentration shifted from negative to positive in the closed and open system, respectively. Among ^{13}C-CPMAS NMR, root damage was negatively correlated with aromatic C fractions both in the open and closed system. Oppositely, weak positive correlation was recorded between O-alkyl and carboxylic C regions with root damage.

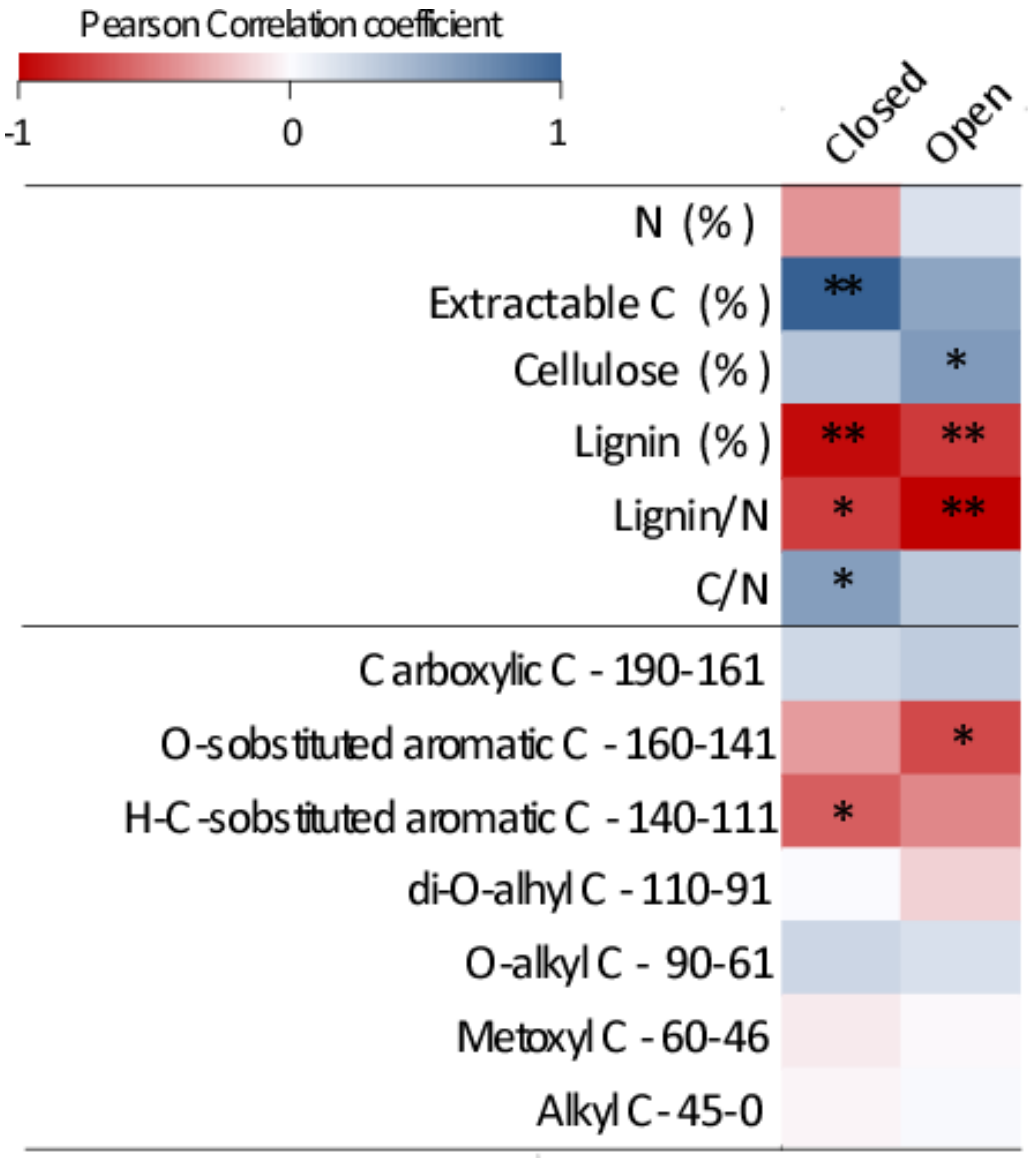

Figure 5.1.6. Heat-plot of correlation (Pearson's r) between *Alnus glutinosa* root damage with litter chemical descriptors in the closed and open experimental system. Asterisks indicate significant correlation values (Pearson's *r*; **, $P < 0.01$; *, $P < 0.05$), either negative (red) or positive (blue).

Self- and heterologous DNA effect on A. glutinosa root

DNA from conspecifics and heterospecific supplied as fragments products by sonication caused a substantial root damage only in closed system, with no difference recorded among treatments with the control in the open system (Figure 5.1.7). In the closed system, the root damage level was significantly higher in presence of self-DNA compared with heterologous DNA.

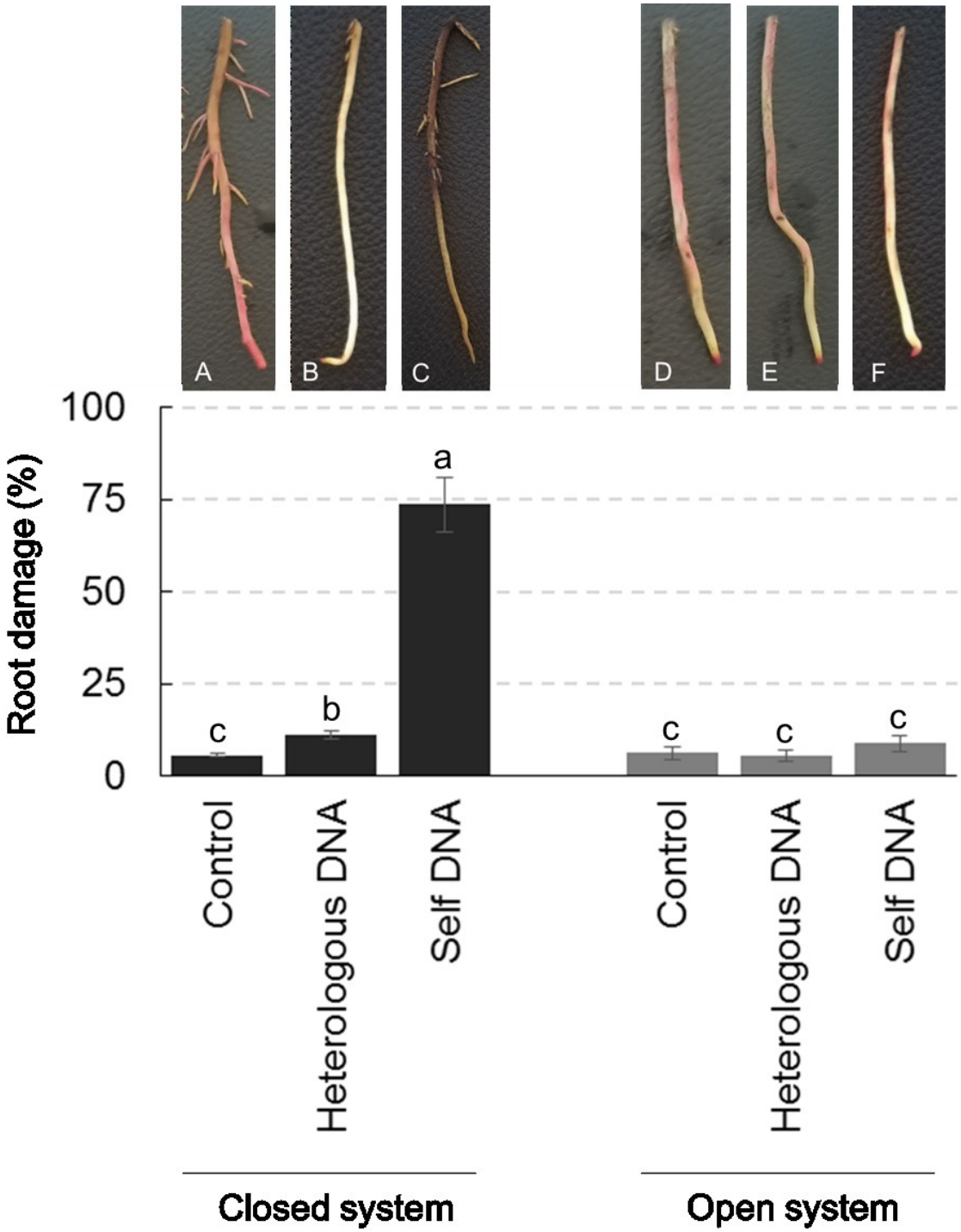

Figure 5.1.7. Effects of heterologous DNA from *Festuca drymeja* and self-DNA of *Alnus glutinosa* on root of *A. glutinosa* after 72 hours of exposition in field condition in open and closed system. Heterologous and self-DNA were applied at concentration of 50 ppm. Pictures are examples of root at the end of the experiment: A: control in closed system; B: heterologous DNA in closed system; C: self-DNA in closed system; D: control in open system; E: heterologous DNA in open system; F: self-DNA in open system. Data are mean and standard deviation; n = 10 for each bar, different letters indicate statistically significant differences (Duncan test, $P<0.05$).

5.1.4. Discussion

Litter effects on aquatic roots

According with a number of laboratory studies (Patrick, 1971; Putnam *et al.*, 1983; Rice, 2012), we found that undecomposed litter caused a severe and acute damage on *Alnus* roots, occurred in the 72 hours of duration of the experiment. Most of previous studies recorded the inhibitory effects of undecomposed litter in laboratory bioassay or, in few cases, in pot experiment where litter was add to soil (Tagliavini & Marangoni, 1992; Van der Putten *et al.*, 1997; Dorrepaal, 2007). Here, we confirm the inhibitory effect of fresh litter, extending these evidences to aquatic roots of a riparian tree. The litter inhibitory effect, however, largely varied with plant species and decomposition time. In detail, *H. helix* was the most toxic litter while *F. drymeja* the least. Moreover, during decomposition the harmful effect almost disappeared for the four tested litter, included the highly inhibitory *A. glutinosa, H. helix*, and *P. nigra*. Previous studies well explained the connection between litter chemistry and their inhibitory effect on plant growth (Bonanomi *et al.* 2011) and, in this regard, the present finding is mostly confirmatory. The initial phase of decomposition mainly consists of plant tissue breakdown, release of cell contents and associated chemical compound that include many compounds ranging from sugar, amino acids, secondary metabolites, and DNA. Evidence from both proximate and ^{13}C-CPMAS NMR data indicate that root damage was correlated with the labile and extractable C fraction, and negatively with lignin and aromatic C compounds. Interestingly, during decomposition all litter types become depleted of the extractable C, being used by decomposed microbiota, and enriched of lignin like compounds that do not exert inhibitory effect on plant roots (Kögel-Knabner, 2002; Preston *et al.*, 2009a)). The importance of extractable C fraction is highlighted by the higher negative correlation found in closed compared to open system. These results suggest that soluble, highly inhibitory compounds that is rapidly released during plant cells breakdown are effectively removed by flowing water, with a minor impact of the lignified and solid fraction of litter that remain in direct contact with the aquatic roots. In this regards, metabolomics studies demonstrated that chemical complexity of leaf litter being composed by hundreds of molecules (Wickings *et al.*, 2012; Wallenstein *et al.*, 2013). The identification of the compound/s responsible of the observed root damage is over the aim of this work, then future research could attempt to understand the major allelopathic molecules and their ecological consequences at in field condition.

Alternatively, root growth inhibition by plant litter and organic amendment has been attributed to N immobilization caused by competition between root and microbes. The N immobilization hypothesis state that organic matter having C/N ratio higher than 30 reduce N availability in a time frame ranging from weeks to years (Zimmerman *et al.*, 1995; Pansu & Thuriès, 2003). This hypothesis is not supported by own work for two reasons. First, all eight tested litter had a C/N ratio below the threshold of 30. In fact, we found a negative correlation in closed system between root damage and N concentration, with *F. drymeja* that was the least toxic despite the higher C/N ratio. Second, we observed extensive root necrosis in a time-frame (i.e. 72 hours) that is too short to allow a substantial N immobilization by microbes (Hodge *et al.*, 2000; Bonanomi *et al.*, 2019). Our data clearly indicate that, at least in our experimental condition and the tested litter, root damage is not caused by a reduced N availability.

Most of previous studies focusing on litter allelopathic effects were heavily criticized because of the use of fast-growing, sensitive target species, having little if any ecological connect with the studied litter. Here, the aquatic roots of *A. glutinosa* appear very sensitive to litter of four coexisting species showing extensive root necrosis after few hours of contact with leaf tissue and the associated dissolved organic fraction. However, assessing the relative species sensitive to different litter type is a challenge task because of the limited studies that systematically addressed this issue. A recent work, based on 14 plants species used as bioassay tested over 36 litter types revealed a species-specific response with annual plants far more sensitive than long-lived woody species (Bonanomi *et al.,* 2017). An early study by (van der Putten, 1997) evaluated the growth of three emergent macrophytes, i.e., *Phragmites australis*, *Typha latifolia*, and *Glyceria maxima* is pot amended with *P. australis* litter. The study revealed that semi-emergent macrophytes were less susceptible to litter inhibitory effect than submerged species i.e. the rank of litter sensitivity was *P. australis* >> *T. latifolia* > *G. maxima*. Negative effect of litter or accumulated organic matter has been reported for macrophytes such as *T. latifolia* (McNaughton, 1968), *Scirpus maritimus* (Clevering & Van der Putten, 1995), *P. australis* (Armstrong & Armstrong, 1999). Moreover, several aquatic plants as *Lemna minor* (Einhellig *et al.*, 1985), *Lepidium sativum* (Gehringer *et al.*, 2003), among many others reviewed in (Mohan & Hosetti, 1999) are routinely used for laboratory bioassay in allelopathy studies because of their sensitivity to organic chemicals. This body of evidence suggest that floating plants, macrophytes, and aquatic roots of riparian tree species are more sensitive to litter phytotoxicity compared with terrestrial plant species. This seems logic in evolutionary terms because aquatic species are not in contact with litter and the associated dissolved organic fractions and, consequently, are not adapted to the specific chemical environment present, for instance, in the floor of litter rich forests. The physiological mechanisms of root adaptation and relative sensitivity of different litter type remain unknown. Then, further studies are needed to test the hypothesis that aquatic plants are more sensitive than terrestrial plant to litter phytotoxicity and to discover the associated molecular and physiological basis.

The finding of high litter sensitivity may have relevant implications for ecosystem functioning in relation to changes in water hydrological regime. Streams, ponds, lagoon, and lake in Mediterranean climate are naturally subject to large fluctuations in water regime because elevated evaporation, a factor that may be exacerbate by climatic changes (Gasith & Resh, 1999). In this context, aquatic root systems could be periodically subject to drying conditions that may create higher concentration of litter and dissolved organic carbon (Vazquez *et al.*, 2011). The periodical occurrence of dry periods during summer will caused litter damage by accumulated litter, with potential negative impact at stand level. In this regard, we acknowledge that our study is based on a single test species and using a single litter application rate and, then, more data are needed to properly appreciate the impact of litter accumulation on aquatic root system functionality. In more general terms, the occurrences of stand die-back of *P. australis* has been associated to litter accumulation and the consequent autotoxic effect on root system, both in temperate (Armstrong & Armstrong, 1999), and Mediterranean climates (Gigante *et al.*, 2011). Recently, stand die-back of several plants in lagoon and saltmarsh has become more frequent and intense (Alber *et al.*, 2008). Future studies are urgently required for an effective

up-scale of the potential effect of litter phytotoxicity and autotoxicity, focusing on the interaction with fluctuation of hydrological regimes.

DNA effects on aquatic roots

In the last decade several experimental evidence demonstrated the inhibitory effect of self DNA on different organisms ranging from bacteria (Berne *et al.*, 2010), algae, fungi, insect and protozoa (Mazzoleni *et al.*, 2015c), up to higher plants (Mazzoleni *et al.*, 2015a; Barbero *et al.*, 2016). However, despite the growing evidence of self DNA toxicity, the underlying cellular and molecular mechanisms are still unknown. In this regards, (Cartenì *et al.*, 2016) proposed the hypothesis that the mixture of self-DNA fragment produced during decomposition of the nuclear genome, may interference or inhibit cell functionality. Beyond the proposed underlying mechanisms, it is evident that all available experimental evidence was obtained under highly controlled laboratory conditions. Here, for the first time, we extend the evidence of self DNA toxicity to filed conditions, opening the question if and in under what circumstances this effect would be relevant in determine plant community structure and functioning. However, the available experimental evidence are consistent with the model proposed by (Mazzoleni *et al.*, 2010) that explain the formation of stable, highly productive monospecific stands only in flowing salt and freshwater condition, irrespectively of the latitudinal level, because of continuous removal of self DNA molecules thanks to its water solubility. Indeed, we are aware that more experimental evidence are required to define the real role of self DNA toxicity in community structure and diversity, also considering that our experiment was carried out with only two DNA types and a single concentration (50 ppm).

5.1.5. Conclusions

In this study we conceived, developed and tested a novel method to assess the effect of leaf litter and self DNA on roots of riparian trees in field condition. We found that leaf litter has an age-dependent effect on *A. glutinosa* root, causing a major inhibitory effect when undecomposed and in the closed system that mimic stagnant water. On the contrary, after decomposition, litter becomes almost harmless even in the closed system lacking water recycling. Litter inhibitory effect was satisfactorily explained by chemical descriptors, with a depletion of extractable C and an enrichment of lignin and recalcitrant compounds during decomposition. In other words, litter rich of extractable C and total N was more toxic compared to more lignified plant tissue. In a second, independent experiment, the novel method demonstrates that self DNA, but not heterologous one, cause an acute toxic effect on *A. glutinosa* root in the closed system. In the open system, a method that mimic the conditions present in streams and rivers with flowing water, the harmful effect of fresh litter as well that of self DNA was dramatically reduced. We are aware that the implications of our findings, for the understanding of litter impact on plant community structure and diversity, are limited by the use of a single target species (i.e. *A. glutinosa*), only eight litter type used at one application rate (25 g L^{-1}) cannot fully represent the variable conditions that can be meet by aquatic roots in real ecosystems. However, we provided for the first time a method that will allow to study the impact of leaf litter as well as of pure chemical compounds in field conditions. Future studies, however, are needed to fully understand the ecological implication of litter accumulation,

and the associated release of dissolved organic fraction, under fluctuating hydrological regimes in causing vegetation stand die-back.

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6. Conclusions and implications in plant community ecology

In the present thesis some particular cases of plant-litter feedback dynamics are described. Here an enlarged point of view of plant-soil feedback generated from litter, is provided including also those ecosystems in which the contribution of soil is minimized by great inputs of water. The effect of phytotoxicity from litter decomposition is explored at conspecific and heterospecific levels. Additionally, the effect of dominant Basidiomycetes, generated by the late stages of litter decomposition, on plant and soil microbiota has been deeply described. Specifically, experimental works, bibliographic reviews and environmental surveys, suggested that the Basidiomycota phylum act likely as a pivotal figure in plant-soil interactions and ecosystem structuration. Mostly, because Basidiomycetes are ecosystem engineer, shaping plant communities either as symbiotic partners of plants and also as free saprotroph in grassland soils. When the presence of the Basidiomycetes is not facilitated by prohibitive conditions (water logged environment), chemical products of litter decomposition dominates the scenario.

6.1. Ectomycorrhizal symbiosis

The experimental section relating ECM symbiosis and litter autotoxicity was aimed to replicate the experimentation conduced in laboratory condition on *Q. ilex* by Mazzoleni *et al* in 2015a in more natural conditions. Comparing *Q. ilex* seedlings with or without mutualistic relationship and a soil matrix we confirmed the effect observed by Mazzoleni and co-workers stating that plant in contact with conspecific litter in late stages of decomposition are characterized by depressed growth as effect of litter autotoxicity. Moreover, as hypothesized, the presence of ectomycorrhizal symbiotic partner permits the plant to avoid the detrimental effect of litter by transform the last in an auto-promotive factor. The advantages provided by the conjunction of conspecific litter and Basidiomycetes symbionts is still undefined. Probably, the advantage that ectomycorrhizal symbiosis provides to plants partner rely on ability of the ectomycorrhizal fungus to scavenge nutrients from litter. Indeed, could not be excluded that phytotoxic molecules released by litter are degraded and reabsorbed by the fungus and in turns retransferred to the plants in form of nutrients. However, the saprobic activity of the Basidiomycetes is dependent by other microbial species in soil as they can unlock resources. Particularly, in absence of these facilitator microbiota, the effect is reflected on plants carrying the symbiosis that showed depressed growth. This is in line with the behavior of C-selected fungi (generally Basidiomycetes) that need a microbial community facilitating the access to resources from organic matter. Particularly for the last sentence, the though the plant are able exploit the strategic ability of Basidiomycetes to became dominant in soil is suggested. This led to indirect implication of litter as driver for this specific shift of strategy from fungal to plant kingdom.

6.2. Relation with water

Excluding soil interactions, the direct effect of litter decomposition by-products in laboratory and natural environments at cellular, histologic and individual levels is demonstrated. Specifically, a generalized phytotoxicity by increased levels of extractable C and total N is observed for litter in aquatic environment. With litter ageing the phytotoxicity shift from generalized to conspecific supporting the self-DNA

autotoxicity theory. In turn, plant communities in water ecosystem tends to be characterized by low diversity because as effect of water removing litter phytotoxicity. In light of this, phenomenon like *P. australis* dieback (Armstrong & Armstrong, 1999) could be induced by limited water exchanges and accumulation of phytotoxic compound in proximity of plant roots. Moreover, Mangrove zonation was explained as the ability of different plants species to colonize seaward coastline by increasing tolerance to salinity gradients. The concept was seen as a successional pattern where a salt tolerant species (in most of the case *Rhizophora* spp.) colonize and consequently decline leaving the place for new mangrove forming species (i.e *Avicennia* spp.) (Snedaker, 1982). However, the same pattern is observed in freshwater riparian ecosystem, with *Salix* and *Alnus* genus as the most proximal to the water corridors, and *Populus*, *Betula* and *Quercus* distributed at increasing distance from the water (Quézel & Médail, 2017). These vegetation patterns, both in saline and fresh water environment, could be explained considering that species with elevated sensibility to phytotoxicity distribute in proximity of water corridors as microenvironment that dilute phytotoxic effect of litter.

6.3. Fairy Rings

The repercussion of microbial dominance on plant community in grassland is demonstrated. The fairy rings patterns are unusual mechanism of plant-soil feedback, this since conditioning effect is produced by litter from the whole community instead of a single species. Decomposition of the litter induce microbial succession until the appearance of a dominant fungal fronts altering the whole structure of soil. Similarly to a disturbance phenomenon, the fungus favor the establishment of a plant community composed by short-lived and fast-growing species because excreting a detrimental effect on the plant community in the grassland external to fairy ring. The effect is specular in telluric microbiota indicating that fairy rings can affect the whole ecosystem structure likely as an ecosystem engineer. Both, Fairy rings of *A. arvensis* and *C. gambosa* showed this effect in the environment in which they persist. Additionally, bibliographic evidences of the effect from fairy rings of *M. oreades* (Cosby, 1960) and, in different biogeographic areas, of *A. arvensis* (Edwards, 1984) supported the idea that the fungus can reshape ecosystem structure and hence species composition. The mechanisms hypothesized to promote plant death in correspondence of fairy rings mycelium has been scrutinized. More probably the effect of Type I fairy rings is given to increased level of hydrophobicity. Indeed, when testing the effect of VOCs, evidences indicated that the release of volatiles compounds are promotive for plant growth and germination. More probably, the stimulant effect of the fungus on plant justify the appearance of verdant belt of vegetation in proximity of the fairy rings (Type 2 fairy rings).

6.4. Evolutive implications of feedbacks mediated by litter.

For what concern the present work, is possible to conclude in a generalized way that litter decomposition byproducts are phytotoxic for plants. This effect can vary according the stages of decomposition of the litter and whether this effect is observed at heterospecific and/or conspecific levels. The phytotoxic effects from litter are demonstrated by the stented growth of non-symbiotic seedlings of *Q. ilex* in soil

enriched with conspecific litter and furtherly confirmed by the a-specific and species-specific effects of litter on roots of *A. glutinosa.* In light of this, is possible to make some speculative consideration at evolutive scale. The loop of interactions known as negative feedback are apparently more present respect to the positive ones (Kulmatiski *et al.*, 2008). Despite this, plants belonging to *Fagaceae, Dipterocarpaceae*, and *Caesalpiniaceae* families are able to overcome these unsuitable conditions by instauration of the symbiosis with basidiomycetes in soil. Could be proposed for future study to consider that mechanisms promoting negative direction of plant-soil feedback act as evolutive driver by selecting plant populations or individuals able to carry the cost of a symbiotic relationship. In other words, is possible that the instauration of negative condition induces reproductive advantages for plants capable to adopt these symbiotic strategies.

In parallel is observed that litter decomposition can promote the formation of microbial communities composed by dominant saprobic basidiomycetes like fairy rings. Interestingly, fairy rings are observed also in woodland ecosystem being originated by ectomycorrhizal plant rootlets (i.e. ECM between *T. matsutake* with *P. densiflora*) (Ohara & Hamada, 1967; Kataoka *et al.*, 2012).ECM symbiosis is more probably originated by the interaction between plants and a saprophytic fungal ancestor, more similar to the actual fairy rings, in opposition to the AM symbionts that are more probably originated by a pathogenic relationship (Jacquemyn & Merckx, 2019). Beside producing a competitive advantage with respect to the whole microbial community in soils, could be argued that the advantage obtained from the late stages of decompositions of litter for the fungus can be transferred to the plants able to carry the symbiosis. As results, the tendencies of the fungus to became dominant is reflected on the plant counterpart that became dominant as well. The most acknowledged way in which the fungus can do this is through its enzymatic arsenal. The availability of strategies able to degrade a wide range of compounds in soil can allow the symbiotic fungi to fragment these compounds at ineffective level by the biological point of view. Indeed, adsorption of nutrients by ECM fungi, to take place, need a degradation through an increase of zymogenous activities targeting biomolecules available in soil. This action by the fungus induce an heavily fragmentation of compounds that are, in turn, immobilized in the mycelium and in the plant symbiotic partner (Bowen, 1973; Marks, 2012; Hawkins *et al.*, 2015). At the same level could be proposed to assess the effective action of these fungi on molecules promoting autotoxicity as in the case of Self-DNA as it could be that DNA is a source of Nitrogen and Phosphorous given its chemical nature. Interestingly, given the suggestion that autotoxicity can induce the formation of ECM symbiosis, could be stressed in future studies whether the application of self-DNA can promote the formation of ECM symbiosis in vitro as is could produce sounding repercussion in restoration ecology and production of valuable foods.

7. Bibliography

1. **Abelho M. 2001.** From litterfall to breakdown in streams: a review. *The Scientific World Journal* **1**: 656-680.
2. **Abesha E, Caetano-Anollés G, Høiland K. 2003.** Population genetics and spatial structure of the fairy ring fungus Marasmius oreades in a Norwegian sand dune ecosystem. *Mycologia* **95**(6): 1021-1031.
3. **Aerts R. 1997a.** Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems. *Oikos* **79**: 439-449.
4. **Aerts R. 1997b.** Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos*: 439-449.
5. **Alber M, Swenson EM, Adamowicz SC, Mendelssohn IA. 2008.** Salt marsh dieback: an overview of recent events in the US. *Estuarine, Coastal and Shelf Science* **80**(1): 1-11.
6. **Almendros G, Knicker H, González-Vila FJ. 2003.** Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state 13C-and 15N-NMR spectroscopy. *Organic Geochemistry* **34**(11): 1559-1568.
7. **Anbarashan M, Parthasarathy N. 2013.** Tree diversity of tropical dry evergreen forests dominated by single or mixed species on the Coromandel coast of India. *Tropical Ecology* **54**(2): 179-190.
8. **Andrews JH, Spear RN, Nordheim EV. 2002.** Population biology of Aureobasidium pullulans on apple leaf surfaces. *Canadian journal of microbiology* **48**(6): 500-513.
9. **Aneja MK, Sharma S, Fleischmann F, Stich S, Heller W, Bahnweg G, Munch JC, Schloter M. 2006.** Microbial colonization of beech and spruce litter—influence of decomposition site and plant litter species on the diversity of microbial community. *Microbial ecology* **52**(1): 127-135.
10. **Angelini P, Tirillini B, Venanzoni R. 2012.** In vitro antifungal activity of Calocybe gambosa extracts against yeasts and filamentous fungi. *African Journal of Microbiology Research* **6**(8): 1810-1814.
11. **Ann MN, Cho YE, Ryu HJ, Kim HT, Park K. 2013.** Growth promotion of tobacco plant by 3-hydroxy-2-butanone from Bacillus vallismortis EXTN-1. *The Korean Journal of Pesticide Science* **17**(4): 388-393.
12. **Antonovics J, Levin DA. 1980.** The ecological and genetic consequences of density-dependent regulation in plants. *Annual review of Ecology and Systematics* **11**(1): 411-452.
13. **Armstrong J, Armstrong W. 1999.** Phragmites die-back: toxic effects of propionic, butyric and caproic acids in relation to pH. *The New Phytologist* **142**(2): 201-217.
14. **Ashton IW, Miller AE, Bowman WD, Suding KN. 2010.** Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* **91**(11): 3252-3260.
15. **Atwater DZ, Ervine C, Barney JN. 2018.** Climatic niche shifts are common in introduced plants. *Nature ecology & evolution* **2**(1): 34.
16. **Ayer WA, Craw PA, Stout TJ, Clardy J. 1989.** Novel sesquiterpenoids from the fairy ring fungus, Marasmius oreades. *Canadian Journal of Chemistry* **67**(5): 773-778.
17. **Bailly A, Groenhagen U, Schulz S, Geisler M, Eberl L, Weisskopf L. 2014.** The inter-kingdom volatile signal indole promotes root development by interfering with auxin signalling. *The Plant Journal* **80**(5): 758-771.
18. **Baldrian P. 2003.** Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial technology* **32**(1): 78-91.
19. **Baldrian P 2008**. Enzymes of saprotrophic basidiomycetes. *British mycological society symposia series*: Elsevier. 19-41.
20. **Baldrian P. 2009.** Ectomycorrhizal fungi and their enzymes in soils: is there enough evidence for their role as facultative soil saprotrophs? *Oecologia* **161**(4): 657-660.
21. **Barbero F, Guglielmotto M, Capuzzo A, Maffei M. 2016.** Extracellular self-DNA (esDNA), but not heterologous plant or insect DNA (etDNA), induces plasma membrane depolarization and calcium signaling in lima bean (Phaseolus lunatus) and maize (Zea mays). *International journal of molecular sciences* **17**(10): 1659.
22. **Bartelt-Ryser J, Joshi J, Schmid B, Brandl H, Balser T. 2005.** Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspectives in plant ecology, evolution and systematics* **7**(1): 27-49.
23. **Battistuzzi F, Hedges S. 2009.** Eubacteria. *The timetree of life (eds SB Hedges & S. Kumar)*: 106-115.

24. **Bäumler AJ, Sperandio V. 2016.** Interactions between the microbiota and pathogenic bacteria in the gut. *Nature* **535**(7610): 85.
25. **Bazzaz F, Pickett S. 1980.** Physiological ecology of tropical succession: a comparative review. *Annual review of Ecology and Systematics* **11**(1): 287-310.
26. **Belsky AJ. 1992.** Effects of grazing, competition, disturbance and fire on species composition and diversity in grassland communities. *Journal of vegetation science* **3**(2): 187-200.
27. **Benítez T, Rincón AM, Limón MC, Codon AC. 2004.** Biocontrol mechanisms of Trichoderma strains. *International microbiology* **7**(4): 249-260.
28. **Benjamini Y, Hochberg Y. 1995.** Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the royal statistical society. Series B (Methodological)*: 289-300.
29. **Bennett JA, Klironomos J. 2019.** Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. *New Phytologist* **222**(1): 91-96.
30. **Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Klironomos J. 2017.** Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* **355**(6321): 181-184.
31. **Berg B, Ekbohm G** McClaugherty C. 2003. Plant litter, decomposition, humus formation, carbon sequestration: Springer, Berlin.
32. **Berg B, McClaugherty C. 2008.** Decomposition as a process. *Plant Litter: Decomposition, Humus Formation, Carbon Sequestration*: 11-33.
33. **Berg B, McClaugherty C 2014.** Decomposition as a process: some main features. *Plant Litter*: Springer, 11-34.
34. **Berne C, Kysela DT, Brun YV. 2010.** A bacterial extracellular DNA inhibits settling of motile progeny cells within a biofilm. *Molecular microbiology* **77**(4): 815-829.
35. **Berni Canani R, De Filippis F, Nocerino R, Laiola M, Paparo L, Calignano A, De Caro C, Coretti L, Chiariotti L, Gilbert JA. 2017.** Specific Signatures of the Gut Microbiota and Increased Levels of Butyrate in Children Treated with Fermented Cow's Milk Containing Heat-Killed Lactobacillus paracasei CBA L74. *Appl Environ Microbiol* **83**(19): 10.
36. **Bever JD, Westover KM, Antonovics J. 1997.** Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*: 561-573.
37. **Bezemer TM, Lawson CS, Hedlund K, Edwards AR, Brook AJ, Igual JM, Mortimer SR, Van Der Putten WH. 2006.** Plant species and functional group effects on abiotic and microbial soil properties and plant–soil feedback responses in two grasslands. *Journal of Ecology* **94**(5): 893-904.
38. **Bhatnagar JM, Peay KG, Treseder KK. 2018.** Litter chemistry influences decomposition through activity of specific microbial functional guilds. *Ecological Monographs*.
39. **Blenis P, Chow P, Duncan I, Knowles N. 2004.** Cyanide levels near fairy rings affect the growth of grasses and fungi. *Canadian Journal of Botany* **82**(9): 1324-1329.
40. **Blenis P, Nadeau L, Knowles N, Logue G. 1997.** Evaluation of fungicides and surfactants for control of fairy rings caused by Marasmius oreades (Bolt ex. Fr.) Fr. *HortScience* **32**(6): 1077-1084.
41. **Boddy L, Hiscox J. 2016.** Fungal ecology: principles and mechanisms of colonization and competition by saprotrophic fungi. *Microbiology spectrum* **4**(6).
42. **Bokulich NA, Mills DA. 2013.** Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. *Appl. Environ. Microbiol.* **79**(8): 2519-2526.
43. **Bonanomi G, Caporaso S, Allegrezza M. 2006a.** Short-term effects of nitrogen enrichment, litter removal and cutting on a Mediterranean grassland. *Acta Oecologica* **30**(3): 419-425.
44. **Bonanomi G, Caporaso S, Allegrezza M. 2009.** Effects of nitrogen enrichment, plant litter removal and cutting on a species-rich Mediterranean calcareous grassland. *Plant Biosystems* **143**(3): 443-455.
45. **Bonanomi G, Cesarano G, Gaglione SA, Ippolito F, Sarker T, Rao MA. 2017a.** Soil fertility promotes decomposition rate of nutrient poor, but not nutrient rich litter through nitrogen transfer. *Plant and soil* **412**(1-2): 397-411.
46. **Bonanomi G, Cesarano G, Lombardi N, Motti R, Scala F, Mazzoleni S, Incerti G. 2017b.** Litter chemistry explains contrasting feeding preferences of bacteria, fungi, and higher plants. *Scientific reports* **7**(1): 9208.

47. **Bonanomi G, De Filippis F, Cesarano G, La Storia A, Zotti M, Mazzoleni S, Incerti G. 2019.** Linking bacterial and eukaryotic microbiota to litter chemistry: Combining next generation sequencing with 13C CPMAS NMR spectroscopy. *Soil Biology and Biochemistry* **129**: 110-121.
48. **Bonanomi G, Del Sorbo G, Mazzoleni S, Scala F. 2007.** Autotoxicity of decaying tomato residues affects susceptibility of tomato to Fusarium wilt. *Journal of Plant Pathology*: 219-226.
49. **Bonanomi G, Giannino F, Mazzoleni S. 2005.** Negative plant–soil feedback and species coexistence. *Oikos* **111**(2): 311-321.
50. **Bonanomi G, Incerti G, Abd El-Gawad AM, Sarker TC, Stinca A, Motti R, Cesarano G, Teobaldelli M, Saulino L, Cona F. 2018a.** Windstorm disturbance triggers multiple species invasion in an urban Mediterranean forest. *iForest-Biogeosciences and Forestry* **11**(1): 64.
51. **Bonanomi G, Incerti G, Abd El-Gawad AM, Cesarano G, Sarker TC, Saulino L, Lanzotti V, Saracino A, Rego FC, Mazzoleni S. 2018b.** Comparing chemistry and bioactivity of burned vs. decomposed plant litter: different pathways but same result? *Ecology* **99**(1): 158-171.
52. **Bonanomi G, Incerti G, Barile E, Capodilupo M, Antignani V, Mingo A, Lanzotti V, Scala F, Mazzoleni S. 2011.** Phytotoxicity, not nitrogen immobilization, explains plant litter inhibitory effects: evidence from solid-state 13C NMR spectroscopy. *New Phytologist* **191**(4): 1018-1030.
53. **Bonanomi G, Incerti G, Cesarano G, Gaglione SA, Lanzotti V. 2015.** Cigarette butt decomposition and associated chemical changes assessed by 13C CPMAS NMR. *PloS one* **10**(1): e0117393.
54. **Bonanomi G, Incerti G, Giannino F, Mingo A, Lanzotti V, Mazzoleni S. 2013.** Litter quality assessed by solid state 13C NMR spectroscopy predicts decay rate better than C/N and Lignin/N ratios. *Soil Biology and Biochemistry* **56**: 40-48.
55. **Bonanomi G, Incerti G, Stinca A, Cartenì F, Giannino F, Mazzoleni S. 2014.** Ring formation in clonal plants. *Community Ecology* **15**(1): 77-86.
56. **Bonanomi G, Mingo A, Incerti G, Mazzoleni S, Allegrezza M. 2012.** Fairy rings caused by a killer fungus foster plant diversity in species-rich grassland. *Journal of vegetation Science* **23**(2): 236-248.
57. **Bonanomi G, Rietkerk M, Dekker SC, Mazzoleni S. 2008.** Islands of fertility induce co-occurring negative and positive plant-soil feedbacks promoting coexistence. *Plant ecology* **197**(2): 207-218.
58. **Bonanomi G, Sicurezza MG, Caporaso S, Esposito A, Mazzoleni S. 2006b.** Phytotoxicity dynamics of decaying plant materials. *New Phytologist* **169**(3): 571-578.
59. **Bongers F, Poorter L, Hawthorne WD, Sheil D. 2009.** The intermediate disturbance hypothesis applies to tropical forests, but disturbance contributes little to tree diversity. *Ecology letters* **12**(8): 798-805.
60. **Bonkowski M. 2004.** Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* **162**(3): 617-631.
61. **Bonnarme P, Jeffries TW. 1990.** Mn (II) regulation of lignin peroxidases and manganese-dependent peroxidases from lignin-degrading white rot fungi. *Appl. Environ. Microbiol.* **56**(1): 210-217.
62. **Boucher DH. 1981.** Seed predation by mammals and forest dominance by Quercus oleoides, a tropical lowland oak. *Oecologia* **49**(3): 409-414.
63. **Bowen G. 1973.** Mineral nutrition of ectomycorrhizae. *Ectomycorrhizae: Their ecology and physiology*: 151-205.
64. **Bridge P, Spooner B. 2001.** Soil fungi: diversity and detection. *Plant and soil* **232**(1-2): 147-154.
65. **Brookshire EJ, Thomas SA. 2013.** Ecosystem consequences of tree monodominance for nitrogen cycling in lowland tropical forest. *PLoS One* **8**(7): e70491.
66. **Brown AJ, Payne CJ, White PS, Peet RK. 2019.** Shade tolerance and mycorrhizal type may influence sapling susceptibility to conspecific negative density dependence. *Journal of Ecology*.
67. **Brundrett M. 1991.** Mycorrhizas in natural ecosystems. *Advances in ecological research* **21**: 171-313.
68. **Brundrett M. 2004.** Diversity and classification of mycorrhizal associations. *Biological reviews* **79**(3): 473-495.
69. **Bulgarelli D, Rott M, Schlaeppi K, van Themaat EVL, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E. 2012.** Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* **488**(7409): 91.
70. **Burnett J, Evans E. 1966.** Genetical Homogeneity and the Stability of the Mating-type Factors of 'Fairy Rings' of Marasmius oreacdes. *Nature* **210**(5043): 1368.

71. **Cadotte MW, Cavender-Bares J, Tilman D, Oakley TH. 2009.** Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PloS one* **4**(5): e5695.
72. **Caesar AJ, Sainju UM, Lartey RT, Gaskin JF. 2013.** Effects of Agaricus lilaceps fairy rings on soil aggregation and microbial community structure in relation to growth stimulation of western wheatgrass (Pascopyrum smithii) in Eastern Montana rangeland. *Microb Ecol* **66**: 120-131.
73. **Callaway RM, Aschehoug ET. 2000.** Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* **290**(5491): 521-523.
74. **Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI. 2010.** QIIME allows analysis of high-throughput community sequencing data. *Nature methods* **7**(5): 335.
75. **Carney KM, Matson PA. 2005.** Plant communities, soil microorganisms, and soil carbon cycling: does altering the world belowground matter to ecosystem functioning? *Ecosystems* **8**(8): 928-940.
76. **Cartenì F, Bonanomi G, Giannino F, Incerti G, Vincenot CE, Chiusano ML, Mazzoleni S. 2016.** Self-DNA inhibitory effects: underlying mechanisms and ecological implications. *Plant signaling & behavior* **11**(4): e1158381.
77. **Cartenì F, Marasco A, Bonanomi G, Mazzoleni S, Rietkerk M, Giannino F. 2012.** Negative plant soil feedback explaining ring formation in clonal plants. *Journal of theoretical biology* **313**: 153-161.
78. **Cartenì F, Sarker TC, Bonanomi G, Cesarano G, Esposito A, Incerti G, Mazzoleni S, Lanzotti V, Giannino F. 2018.** Linking plant phytochemistry to soil processes and functions: the usefulness of 13 C NMR spectroscopy. *Phytochemistry Reviews*: 1-18.
79. **Carvalho LM, Correia PM, Martins-Loução MA. 2004.** Arbuscular mycorrhizal fungal propagules in a salt marsh. *Mycorrhiza* **14**(3): 165-170.
80. **Caspar J, Spiteller P. 2015.** A free cyanohydrin as arms and armour of Marasmius oreades. *ChemBioChem* **16**(4): 570-573.
81. **Catford JA, Daehler CC, Murphy HT, Sheppard AW, Hardesty BD, Westcott DA, Rejmánek M, Bellingham PJ, Pergl J, Horvitz CC. 2012.** The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. *Perspectives in Plant Ecology, Evolution and Systematics* **14**(3): 231-241.
82. **Cesarano G, Incerti G, Bonanomi G. 2016.** The influence of plant litter on soil water repellency: insight from 13C NMR spectroscopy. *PloS one* **11**(3): e0152565.
83. **Cesarano G, Zotti M, Antignani V, Marra R, Scala F, Bonanomi G. 2017a.** SOIL SICKNESS AND NEGATIVE PLANT-SOIL FEEDBACK: A REAPPRAISAL OF HYPOTHESES. *Journal of Plant Pathology* **99**(3).
84. **Cesarano G, Zotti M, Antignani V, Marra R, Scala F, Bonanomi G. 2017b.** Soil sickness and negative plant-soil feedback: A reappraisal of hypotheses. *Journal of Plant Pathology* **99**(3): 545-570.
85. **Chapman SK, Newman GS, Hart SC, Schweitzer JA, Koch GW. 2013.** Leaf litter mixtures alter microbial community development: mechanisms for non-additive effects in litter decomposition. *PloS one* **8**(4): e62671.
86. **Chesson P. 2000.** Mechanisms of maintenance of species diversity. *Annual review of Ecology and Systematics* **31**(1): 343-366.
87. **Choi J-H, Abe N, Tanaka H, Fushimi K, Nishina Y, Morita A, Kiriiwa Y, Motohashi R, Hashizume D, Koshino H. 2010.** Plant-growth regulator, imidazole-4-carboxamide, produced by the fairy ring forming fungus Lepista sordida. *Journal of agricultural and food chemistry* **58**(18): 9956-9959.
88. **Choi J-H, Kikuchi A, Pumkaeo P, Hirai H, Tokuyama S, Kawagishi H. 2016.** Bioconversion of AHX to AOH by resting cells of Burkholderia contaminans CH-1. *Bioscience, biotechnology, and biochemistry* **80**(10): 2045-2050.
89. **Choi J-H, Matsuzaki N, Wu J, Kotajima M, Hirai H, Kondo M, Asakawa T, Inai M, Ouchi H, Kan T. 2019.** Ribosides and Ribotide of a Fairy Chemical, Imidazole-4-carboxamide, as Its Metabolites in Rice. *Organic letters* **21**(19): 7841-7845.
90. **Choi J-H, Wu J, Sawada A, Takeda S, Takemura H, Yogosawa K, Hirai H, Kondo M, Sugimoto K, Asakawa T. 2017.** N-Glucosides of fairy chemicals, 2-azahypoxanthine and 2-aza-8-oxohypoxanthine, in rice. *Organic letters* **20**(1): 312-314.

91. **Choi JH, Fushimi K, Abe N, Tanaka H, Maeda S, Morita A, Hara M, Motohashi R, Matsunaga J, Eguchi Y. 2010.** Disclosure of the "Fairy" of Fairy-Ring-Forming Fungus Lepista sordida. *ChemBioChem* **11**(10): 1373-1377.
92. **Choi JH, Ohnishi T, Yamakawa Y, Takeda S, Sekiguchi S, Maruyama W, Yamashita K, Suzuki T, Morita A, Ikka T. 2014.** The Source of "Fairy Rings": 2-Azahypoxanthine and its Metabolite Found in a Novel Purine Metabolic Pathway in Plants. *Angewandte Chemie International Edition* **53**(6): 1552-1555.
93. **Chou C-H, Patrick Z. 1976.** Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. *Journal of Chemical Ecology* **2**(3): 369-387.
94. **Chuyong G, Newbery D, Songwe N. 2000.** Litter nutrients and retranslocation in a central African rain forest dominated by ectomycorrhizal trees. *New Phytologist* **148**(3): 493-510.
95. **Clark DA, Clark DB. 1984.** Spacing dynamics of a tropical rain forest tree: evaluation of the Janzen-Connell model. *The American Naturalist* **124**(6): 769-788.
96. **Clarke K, Gorley R. 2015.** Getting started with PRIMER v7. *PRIMER-E: Plymouth, Plymouth Marine Laboratory*.
97. **Clevering OA, Van der Putten WH. 1995.** Effects of detritus accumulation on the growth of Scirpus maritimus under greenhouse conditions. *Canadian Journal of Botany* **73**(6): 852-861.
98. **Comita LS, Muller-Landau HC, Aguilar S, Hubbell SP. 2010.** Asymmetric density dependence shapes species abundances in a tropical tree community. *Science* **329**(5989): 330-332.
99. **Conn C, Dighton J. 2000.** Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. *Soil Biology and Biochemistry* **32**(4): 489-496.
100. **Connell JH, Lowman MD. 1989.** Low-diversity tropical rain forests: some possible mechanisms for their existence. *The American Naturalist* **134**(1): 88-119.
101. **Cornwell WK, Cornelissen JH, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindeguy N. 2008.** Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology letters* **11**(10): 1065-1071.
102. **Corrales A, Henkel TW, Smith ME. 2018.** Ectomycorrhizal associations in the tropics–biogeography, diversity patterns and ecosystem roles. *New Phytologist* **220**(4): 1076-1091.
103. **Corrales A, Mangan SA, Turner BL, Dalling JW. 2016.** An ectomycorrhizal nitrogen economy facilitates monodominance in a neotropical forest. *Ecology letters* **19**(4): 383-392.
104. **Cosby H. 1960.** Rings on The Range. *Rangeland Ecology & Management/Journal of Range Management Archives* **13**(6): 283-288.
105. **Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E. 2013.** The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology* **19**(4): 988-995.
106. **Couch H. 1995.** Diseases of Turfgrasses caused by Fungi. *Diseases of Turfgrasses. 3rd ed. Malabar, FL: Krieger Publishing Company. p* **21**: 199.
107. **Cox PA. 1981.** Niche partitioning between sexes of dioecious plants. *The American Naturalist* **117**(3): 295-307.
108. **Cuevas E, Medina E. 1988.** Nutrient dynamics within Amazonian forests. *Oecologia* **76**(2): 222-235.
109. **Czaran T, Bartha S. 1992.** Spatiotemporal dynamic models of plant populations and communities. *Trends in ecology & evolution* **7**(2): 38-42.
110. **Dabral A, Butola B, Khanduri V, Sharma C, Dhanush C. 2018.** Autotoxicity against seed germination, seedling emergence of Quercus leucotrichophora A. Camus Ex. Bahadur. *Journal of Pharmacognosy and Phytochemistry* **7**(5): 1813-1816.
111. **David TS, Henriques M, Kurz-Besson C, Nunes J, Valente F, Vaz M, Pereira J, Siegwolf R, Chaves M, Gazarini L. 2007.** Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. *Tree physiology* **27**(6): 793-803.
112. **Davidson EA, Hart SC, Firestone MK. 1992.** Internal cycling of nitrate in soils of a mature coniferous forest. *Ecology* **73**(4): 1148-1156.

113. **Davidson F, Sleeman B, Rayner A, Crawford J, Ritz K. 1996a.** Context-dependent macroscopic patterns in growing and interacting mycelial networks. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **263**(1372): 873-880.
114. **Davidson F, Sleeman B, Rayner A, Crawford J, Ritz K. 1996b.** Large-scale behavior of fungal mycelia. *Mathematical and Computer Modelling* **24**(10): 81-87.
115. **De Filippis F, La Storia A, Blaiotta G. 2017.** Monitoring the mycobiota during Greco di Tufo and Aglianico wine fermentation by 18S rRNA gene sequencing. *Food microbiology* **63**: 117-122.
116. **De Filippis F, Vitaglione P, Cuomo R, Berni Canani R, Ercolini D. 2018.** Dietary Interventions to Modulate the Gut Microbiome—How Far Away Are We From Precision Medicine. *Inflammatory bowel diseases*.
117. **De Luis M, Raventós J, González-Hidalgo JC. 2006.** Post-fire vegetation succession in Mediterranean gorse shrublands. *Acta Oecologica* **30**(1): 54-61.
118. **De Micco V, Aronne G. 2010.** Root structure of Rumex scutatus growing on slopes. *IAWA journal* **31**(1): 13-28.
119. **Deacon JW. 2013.** *Fungal biology*: John Wiley & Sons.
120. **DeBell DS. 1971.** Phytotoxic effects of cherrybark oak. *Forest Science* **17**(2): 180-185.
121. **Demenois J, Ibanez T, Read J, Carriconde F. 2017.** Comparison of two monodominant species in New Caledonia: floristic diversity and ecological strategies of Arillastrum gummiferum (Myrtaceae) and Nothofagus aequilateralis (Nothofagaceae) rainforests. *Australian Journal of Botany* **65**(1): 11-21.
122. **Dengler J, Bergmeier E, Willner W, Chytrý M. 2013.** Towards a consistent classification of European grasslands. *Applied Vegetation Science* **16**(3): 518-520.
123. **Dennis C, Webster J. 1971.** Antagonistic properties of species-groups of Trichoderma: II. Production of volatile antibiotics. *Transactions of the British Mycological Society* **57**(1): 41-IN44.
124. **Dickie IA, Xu B, Koide RT. 2002.** Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* **156**(3): 527-535.
125. **Dickinson C. 1979.** Fairy rings in Norfolk. *Bulletin of the British Mycological Society* **13**(2): 91-94.
126. **Dighton J. 2007.** 16 Nutrient Cycling by Saprotrophic Fungi in Terrestrial Habitats. *Environmental and microbial relationships* **4**: 287.
127. **Dighton J. 2016.** *Fungi in ecosystem processes*: CRC Press.
128. **Dittmann J, Heyser W, Bücking H. 2002.** Biodegradation of aromatic compounds by white rot and ectomycorrhizal fungal species and the accumulation of chlorinated benzoic acid in ectomycorrhizal pine seedlings. *Chemosphere* **49**(3): 297-306.
129. **Dix NJ. 2012.** *Fungal ecology*: Springer Science & Business Media.
130. **Dix NJ, Webster J 1995.** Colonization and decay of wood. *Fungal Ecology*: Springer, 145-171.
131. **Dodds WK, Gido K, Whiles MR, Fritz KM, Matthews WJ. 2004.** Life on the edge: the ecology of Great Plains prairie streams. *BioScience* **54**(3): 205-216.
132. **Doerr SH. 1998.** On standardizing the 'water drop penetration time'and the 'molarity of an ethanol droplet'techniques to classify soil hydrophobicity: a case study using medium textured soils. *Earth Surface Processes and Landforms: The Journal of the British Geomorphological Group* **23**(7): 663-668.
133. **Dorrepaal E. 2007.** Are plant growth-form-based classifications useful in predicting northern ecosystem carbon cycling feedbacks to climate change? *Journal of Ecology* **95**(6): 1167-1180.
134. **Dowson C, Rayner A, Boddy L. 1989.** Spatial dynamics and interactions of the woodland fairy ring fungus, Clitocybe nebularis. *New Phytologist* **111**(4): 699-705.
135. **Duke SO. 2003.** Ecophysiological aspects of allelopathy. *Planta* **217**(4): 529-539.
136. **Duran-Flores D, Heil M. 2018.** Extracellular self-DNA as a damage-associated molecular pattern (DAMP) that triggers self-specific immunity induction in plants. *Brain, behavior, and immunity* **72**: 78-88.
137. **Ebenye M, Helvyne C, Taudière A, Niang N, Ndiaye C, Sauve M, Awana NO, Verbeken M, De Kesel A, Séne S. 2017.** Ectomycorrhizal fungi are shared between seedlings and adults in a monodominant Gilbertiodendron dewevrei rain forest in Cameroon. *Biotropica* **49**(2): 256-267.
138. **Edwards P. 1984.** The growth of fairy rings of Agaricus arvensis and their effect upon grassland vegetation and soil. *The Journal of Ecology*: 505-513.
139. **Edwards P. 1988.** Effects of the fairy ring fungus Agaricus arvensis on nutrient availability in grassland. *New Phytologist* **110**(3): 377-381.

140. **Ehrenfeld JG, Ravit B, Elgersma K. 2005.** Feedback in the plant-soil system. *Annu. Rev. Environ. Resour.* **30**: 75-115.
141. **Einhellig FA, Leather GR, Hobbs LL. 1985.** Use ofLemna minor L. as a bioassay in allelopathy. *Journal of Chemical Ecology* **11**(1): 65-72.
142. **Elliott JSB. 1926.** Concerning "fairy rings" in pastures. *Annals of Applied Biology* **13**(2): 277-288.
143. **Elliott ML, Hickman RB, Hopkins M. 2002.** Flutolanil for control of necrotic fairy rings on bermudagrass putting greens. *HortTechnology* **12**(4): 656-659.
144. **Ercolini D, De Filippis F, La Storia A, Iacono M. 2012.** "Remake" by high-throughput sequencing of the microbiota involved in the production of water buffalo mozzarella cheese. *Applied and environmental microbiology* **78**(22): 8142-8145.
145. **Espeland E, Caesar AJ, Sainju UM, Lartey RT, Gaskin JF. 2013.** Effects of Agaricus lilaceps fairy rings on soil aggregation and microbial community structure in relation to growth stimulation of western wheatgrass (Pascopyrum smithii) in Eastern Montana rangeland. *Microbial ecology* **66**(1): 120-131.
146. **Evershed H 1884**. Fairy rings: Nature Publishing Group.
147. **Facelli JM, Pickett ST. 1991.** Plant litter: its dynamics and effects on plant community structure. *The Botanical Review* **57**(1): 1-32.
148. **Fidanza M. 2007.** New insight on fairy ring. *Golf Course Management* **75**(3): 107-110.
149. **Fidanza MA, Cisar J, Kostka S, Gregos J, Schlossberg M, Franklin M. 2007.** Preliminary investigation of soil chemical and physical properties associated with type-I fairy ring symptoms in turfgrass. *Hydrological Processes: An International Journal* **21**(17): 2285-2290.
150. **Fierer N. 2017.** Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature reviews microbiology* **15**(10): 579.
151. **Filer Tj. 1965.** Parasitic aspects of a fairy ring fungus, Marasmius oreades. *Phytopathology 55 (10): 1132-1134* **55**(10): 1132-1134.
152. **Finke DL, Snyder WE. 2008.** Niche partitioning increases resource exploitation by diverse communities. *Science* **321**(5895): 1488-1490.
153. **Finkelstein RR, Gibson SI. 2002.** ABA and sugar interactions regulating development: cross-talk or voices in a crowd? *Current opinion in plant biology* **5**(1): 26-32.
154. **Fiore-Donno AM, Weinert J, Wubet T, Bonkowski M. 2016.** Metacommunity analysis of amoeboid protists in grassland soils. *Scientific reports* **6**: 19068.
155. **Fisher R. 1977.** Nitrogen and phosphorus mobilization by the fairy ring fungus, Marasmius oreades (Bolt.) Fr. *Soil Biology and Biochemistry* **9**(4): 239-241.
156. **Fox JF. 1981.** Intermediate levels of soil disturbance maximize alpine plant diversity. *Nature* **293**(5833): 564-565.
157. **Fox JW. 2013.** The intermediate disturbance hypothesis should be abandoned. *Trends in ecology & evolution* **28**(2): 86-92.
158. **Fragniere Y, Bétrisey S, Cardinaux L, Stoffel M, Kozlowski G. 2015.** Fighting their last stand? A global analysis of the distribution and conservation status of gymnosperms. *Journal of Biogeography* **42**(5): 809-820.
159. **Frankland JC. 1998.** Fungal succession—unravelling the unpredictable. *Mycological research* **102**(1): 1-15.
160. **Frey-Klett P, Garbaye Ja, Tarkka M. 2007.** The mycorrhiza helper bacteria revisited. *New Phytologist* **176**(1): 22-36.
161. **Fukami T, Nakajima M, Fortunel C, Fine PV, Baraloto C, Russo SE, Peay KG. 2017.** Geographical variation in community divergence: insights from tropical forest monodominance by ectomycorrhizal trees. *The American Naturalist* **190**(S1): S000-S000.
162. **Gadd G, Watkinson SC, Dyer PS. 2007.** *Fungi in the Environment*: Cambridge University Press.
163. **Garbaye J. 1994.** Tansley review no. 76 helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytologist* **128**(2): 197-210.
164. **Garbaye J, Churin J-L, Duponnois R. 1992.** Effects of substrate sterilization, fungicide treatment, and mycorrhization helper bacteria on ectomycorrhizal formation of pedunculate oak (Quercus robur) inoculated with Laccaria laccata in two peat bare-root nurseries. *Biology and Fertility of Soils* **13**(1): 55-57.

165. **Gartner TB, Cardon ZG. 2004.** Decomposition dynamics in mixed-species leaf litter. *Oikos* **104**(2): 230-246.
166. **Gasith A, Resh VH. 1999.** Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annual review of Ecology and Systematics* **30**(1): 51-81.
167. **Gast RJ, Ledee DR, Fuerst PA, Byers TJ. 1996.** Subgenus systematics of Acanthamoeba: four nuclear 18S rDNA sequence types. *Journal of Eukaryotic Microbiology* **43**(6): 498-504.
168. **Gaston KJ. 2000.** Global patterns in biodiversity. *Nature* **405**(6783): 220.
169. **Gehringer MM, Kewada V, Coates N, Downing TG. 2003.** The use of Lepidium sativum in a plant bioassay system for the detection of microcystin-LR. *Toxicon* **41**(7): 871-876.
170. **Geisen S, Fiore-Donno AM, Walochnik J, Bonkowski M. 2014.** Acanthamoeba everywhere: high diversity of Acanthamoeba in soils. *Parasitology research* **113**(9): 3151-3158.
171. **Gessner MO 2005.** Proximate lignin and cellulose. *Methods to study litter decomposition*: Springer, 115-120.
172. **Gigante D, Venanzoni R, Zuccarello V. 2011.** Reed die-back in southern Europe? A case study from Central Italy. *Comptes Rendus Biologies* **334**(4): 327-336.
173. **González-Pérez JA, González-Vila FJ, Almendros G, Knicker H. 2004.** The effect of fire on soil organic matter—a review. *Environment international* **30**(6): 855-870.
174. **Govan JR. 2000.** Burkholderia cepacia-friend and foe. *Asm News* **66**: 124-125.
175. **Grace JB. 1999.** The factors controlling species density in herbaceous plant communities: an assessment. *Perspectives in plant ecology, evolution and systematics* **2**(1): 1-28.
176. **Gramss G, Voigt KD, Bergmann H. 2005.** Factors influencing water solubility and plant availability of mineral compounds in the tripartite fairy rings of Marasmius oreades (Bolt.: Fr.) Fr. *Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms* **45**(1): 41-54.
177. **Gregory P. 1982.** Fairy rings; free and tethered. *Bulletin of the British Mycological Society* **16**(2): 161-163.
178. **Griffin D. 1963.** Soil moisture and the ecology of soil fungi. *Biological Reviews* **38**(2): 141-166.
179. **Gross ND, Torti SD, Feener Jr DH, Coley PD. 2000.** Monodominance in an African rain forest: Is reduced herbivory important? *Biotropica* **32**(3): 430-439.
180. **Habel JC, Dengler J, Janišová M, Török P, Wellstein C, Wiezik M. 2013.** European grassland ecosystems: threatened hotspots of biodiversity. *Biodiversity and Conservation* **22**(10): 2131-2138.
181. **Halisky P, Peterson J. 1970.** Basidiomycetes associated with fairy rings in turf. *Bulletin of the torrey botanical club*: 225-227.
182. **Hardin G. 1960.** The competitive exclusion principle. *Science* **131**(3409): 1292-1297.
183. **Hardwick N, Heard A. 1978.** The effect of Marasmius oreades in pasture. *Plant Pathology* **27**(2): 53-57.
184. **Harper J, Webster J. 1964.** An experimental analysis of the coprophilous fungus succession. *Transactions of the British Mycological Society* **47**(4): 511-530.
185. **Harrison RD. 2005.** Figs and the diversity of tropical rainforests. *AIBS Bulletin* **55**(12): 1053-1064.
186. **Hart TB, Hart JA, Murphy PG. 1989.** Monodominant and species-rich forests of the humid tropics: causes for their co-occurrence. *The American Naturalist* **133**(5): 613-633.
187. **Hättenschwiler S, Tiunov AV, Scheu S. 2005.** Biodiversity and litter decomposition in terrestrial ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **36**: 191-218.
188. **Hawkins B, Jones MD, Kranabetter J. 2015.** Ectomycorrhizae and tree seedling nitrogen nutrition in forest restoration. *New Forests* **46**(5-6): 747-771.
189. **Hearst C, Nelson D, McCollum G, Sharma S, Rao JR. 2013.** Forest fairy ring fungi Clitocybe nebularis, soil Bacillus spp., and plant extracts exhibit in vitro antagonism on dieback Phytophthora species. *Natural Resources* **4**(02): 189.
190. **Heberle H, Meirelles GV, da Silva FR, Telles GP, Minghim R. 2015.** InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC bioinformatics* **16**(1): 169.
191. **Hedges SB, Blair JE, Venturi ML, Shoe JL. 2004.** A molecular timescale of eukaryote evolution and the rise of complex multicellular life. *BMC evolutionary biology* **4**(1): 2.
192. **Henkel TW. 2003.** Monodominance in the ectomycorrhizal Dicymbe corymbosa (Caesalpiniaceae) from Guyana. *Journal of Tropical Ecology* **19**(4): 417-437.

193. **Henkel TW, Mayor JR, Woolley LP. 2005.** Mast fruiting and seedling survival of the ectomycorrhizal, monodominant Dicymbe corymbosa (Caesalpiniaceae) in Guyana. *New Phytologist* **167**(2): 543-556.
194. **Henkel TW, Terborgh J, Vilgalys RJ. 2002.** Ectomycorrhizal fungi and their leguminous hosts in the Pakaraima Mountains of Guyana. *Mycological Research* **106**(5): 515-531.
195. **Hiscox J, O'Leary J, Boddy L. 2018.** Fungus wars: basidiomycete battles in wood decay. *Studies in mycology* **89**: 117-124.
196. **Hobbie SE. 2015.** Plant species effects on nutrient cycling: revisiting litter feedbacks. *Trends in ecology & evolution* **30**(6): 357-363.
197. **Hodge A, Robinson D, Fitter A. 2000.** Are microorganisms more effective than plants at competing for nitrogen? *Trends in plant science* **5**(7): 304-308.
198. **Hoekstra JM, Boucher TM, Ricketts TH, Roberts C. 2005.** Confronting a biome crisis: global disparities of habitat loss and protection. *Ecology letters* **8**(1): 23-29.
199. **Holmes A, Govan J, Goldstein R. 1998.** Agricultural use of Burkholderia (Pseudomonas) cepacia: a threat to human health? *Emerging infectious diseases* **4**(2): 221.
200. **Howard DH. 1999.** Acquisition, transport, and storage of iron by pathogenic fungi. *Clinical Microbiology Reviews* **12**(3): 394-404.
201. **Hudson H. 1968.** The ecology of fungi on plant remains above the soil. *New Phytologist* **67**(4): 837-874.
202. **Huston MA. 2014.** Disturbance, productivity, and species diversity: empiricism vs. logic in ecological theory. *Ecology* **95**(9): 2382-2396.
203. **Hyatt LA, Rosenberg MS, Howard TG, Bole G, Fang W, Anastasia J, Brown K, Grella R, Hinman K, Kurdziel JP. 2003.** The distance dependence prediction of the Janzen-Connell hypothesis: a meta-analysis. *Oikos* **103**(3): 590-602.
204. **Incerti G, Bonanomi G, Giannino F, Cartenì F, Spaccini R, Mazzei P, Piccolo A, Mazzoleni S. 2017.** OMDY: a new model of organic matter decomposition based on biomolecular content as assessed by 13 C-CPMAS-NMR. *Plant and soil* **411**(1-2): 377-394.
205. **Incerti G, Cartenì F, Cesarano G, Sarker TC, Abd El-Gawad AM, D'Ascoli R, Bonanomi G, Giannino F. 2018.** Faster N release, but not C loss, from leaf litter of invasives compared to native species in Mediterranean ecosystems. *Frontiers in plant science* **9**: 534.
206. **Ingold C. 1974.** Growth and death of a fairy ring. *Bulletin of the British Mycological Society* **8**(2): 74-75.
207. **Jacquemyn H, Merckx VS. 2019.** Mycorrhizal symbioses and the evolution of trophic modes in plants. *Journal of Ecology*.
208. **Janzen DH. 1970.** Herbivores and the number of tree species in tropical forests. *The American Naturalist* **104**(940): 501-528.
209. **Jensen K, Gutekunst K. 2003.** Effects of litter on establishment of grassland plant species: the role of seed size and successional status. *Basic and Applied Ecology* **4**(6): 579-587.
210. **Jiménez-Alfaro B, Silveira FA, Fidelis A, Poschlod P, Commander LE. 2016.** Seed germination traits can contribute better to plant community ecology. *Journal of vegetation Science* **27**(3): 637-645.
211. **Johnson JM, Ludwig A, Furch AC, Mithöfer A, Scholz S, Reichelt M, Oelmüller R. 2019.** The Beneficial Root-Colonizing Fungus Mortierella hyalina Promotes the Aerial Growth of Arabidopsis and Activates Calcium-Dependent Responses That Restrict Alternaria brassicae–Induced Disease Development in Roots. *Molecular Plant-Microbe Interactions*: MPMI-05-18-0115-R.
212. **Jones CG, Lawton JH, Shachak M. 1997.** Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* **78**(7): 1946-1957.
213. **Jumpponen A, Jones K. 2009.** Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate Quercus macrocarpa phyllosphere. *New Phytologist* **184**(2): 438-448.
214. **Kardol P, Martijn Bezemer T, Van Der Putten WH. 2006.** Temporal variation in plant–soil feedback controls succession. *Ecology letters* **9**(9): 1080-1088.
215. **Karst N, Dralle D, Thompson S. 2016.** Spiral and rotor patterns produced by fairy ring fungi. *PloS one* **11**(3): e0149254.
216. **Kataoka R, Siddiqui ZA, Kikuchi J, Ando M, Sriwati R, Nozaki A, Futai K. 2012.** Detecting nonculturable bacteria in the active mycorrhizal zone of the pine mushroom Tricholoma matsutake. *The Journal of Microbiology* **50**(2): 199-206.

217. **Kawagishi H. 2018.** Fairy chemicals–a candidate for a new family of plant hormones and possibility of practical use in agriculture. *Bioscience, biotechnology, and biochemistry* **82**(5): 752-758.
218. **Kawagishi H. 2019.** Are fairy chemicals a new family of plant hormones? *Proceedings of the Japan Academy, Series B* **95**(1): 29-38.
219. **Keiblinger KM, Schneider T, Roschitzki B, Schmid E, Eberl L, Hämmerle I, Leitner S, Richter A, Wanek W, Riedel K. 2012.** Effects of stoichiometry and temperature perturbations on beech leaf litter decomposition, enzyme activities and protein expression. *Biogeosciences* **9**(11): 4537-4551.
220. **Keller NP, Turner G, Bennett JW. 2005.** Fungal secondary metabolism—from biochemistry to genomics. *Nature reviews microbiology* **3**(12): 937.
221. **Kim M, Yoon H, Kim Y, Kim Y, Kong W, Kim J. 2014.** Comparative analysis of bacterial diversity and communities inhabiting the fairy ring of Tricholoma matsutake by barcoded pyrosequencing. *Journal of applied microbiology* **117**(3): 699-710.
222. **Kim M, Yoon HJ, You YH, Kim YE, Woo JR, Seo YG, Lee GM, Kim YJ, Kong WS, Kim JG. 2013.** Metagenomic analysis of fungal communities inhabiting the fairy ring zone of Tricholoma matsutake. *Journal of microbiology and biotechnology* **23**(10): 1347-1356.
223. **Klironomos J, Zobel M, Tibbett M, Stock WD, Rillig MC, Parrent JL, Moora M, Koch AM, Facelli JM, Facelli E. 2011.** Forces that structure plant communities: quantifying the importance of the mycorrhizal symbiosis. *New Phytologist* **189**(2): 366-370.
224. **Klironomos JN. 2002.** Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* **417**(6884): 67.
225. **Kögel-Knabner I. 2002.** The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry* **34**(2): 139-162.
226. **Kominoski JS, Pringle CM, Ball BA, Bradford M, Coleman D, Hall D, Hunter M. 2007.** Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology* **88**(5): 1167-1176.
227. **Kosman DJ. 2003.** Molecular mechanisms of iron uptake in fungi. *Molecular microbiology* **47**(5): 1185-1197.
228. **Kozlowski TT. 2012.** *Fire and ecosystems*: Elsevier.
229. **Kraus TE, Dahlgren RA, Zasoski RJ. 2003.** Tannins in nutrient dynamics of forest ecosystems-a review. *Plant and Soil* **256**(1): 41-66.
230. **Kubicek C, Mach R, Peterbauer C, Lorito M. 2001.** Trichoderma: from genes to biocontrol. *Journal of Plant Pathology*: 11-23.
231. **Kulmatiski A, Beard KH, Stevens JR, Cobbold SM. 2008.** Plant–soil feedbacks: a meta-analytical review. *Ecology letters* **11**(9): 980-992.
232. **Kutschera U. 2007.** Plant-associated methylobacteria as co-evolved phytosymbionts: a hypothesis. *Plant signaling & behavior* **2**(2): 74-78.
233. **Laliberté E, Lambers H, Burgess TI, Wright SJ. 2015.** Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytologist* **206**(2): 507-521.
234. **Lane BG. 1991.** Cellular desiccation and hydration: developmentally regulated proteins, and the maturation and germination of seed embryos. *The FASEB journal* **5**(14): 2893-2901.
235. **Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, Knight R. 2013.** Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature biotechnology* **31**(9): 814.
236. **Lebeau J, Hawn E. 1963.** A simple method for the control of fairy ring caused by Marasmius oreades. *The Journal of the Sports Turf Research Institute* **11**: 23-25.
237. **Leff JW, Bardgett RD, Wilkinson A, Jackson BG, Pritchard WJ, Long JR, Oakley S, Mason KE, Ostle NJ, Johnson D. 2018.** Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *The ISME journal*: 1.
238. **Leigh Jr EG, Davidar P, Dick CW, Puyravaud J-P, Terborgh J, ter Steege H, Wright SJ. 2004.** Why do some tropical forests have so many species of trees? *Biotropica* **36**(4): 447-473.
239. **Li Z-H, Wang Q, Ruan X, Pan C-D, Jiang D-A. 2010.** Phenolics and plant allelopathy. *Molecules* **15**(12): 8933-8952.

240. **Lian C, Narimatsu M, Nara K, Hogetsu T. 2006.** Tricholoma matsutake in a natural Pinus densiflora forest: correspondence between above-and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities. *New Phytologist* **171**(4): 825-836.
241. **Lindahl BD, Finlay RD, Cairney JW. 2005.** Enzymatic activities of mycelia in mycorrhizal fungal communities. *MYCOLOGY SERIES* **23**: 331.
242. **Lodge D. 1989.** The influence of soil moisture and flooding on formation of VA-endo-and ectomycorrhizae in Populus and Salix. *Plant and Soil* **117**(2): 243-253.
243. **Lopez-Iglesias B, Olmo M, Gallardo A, Villar R. 2014.** Short-term effects of litter from 21 woody species on plant growth and root development. *Plant and Soil* **381**(1-2): 177-191.
244. **Loydi A, Eckstein RL, Otte A, Donath TW. 2013.** Effects of litter on seedling establishment in natural and semi-natural grasslands: a meta-analysis. *Journal of Ecology* **101**(2): 454-464.
245. **Lummer D, Scheu S, Butenschoen O. 2012.** Connecting litter quality, microbial community and nitrogen transfer mechanisms in decomposing litter mixtures. *Oikos* **121**(10): 1649-1655.
246. **Macías FA, Simonet AM, Pacheco PC, Barrero AF, Cabrera E, Jiménez-González D. 2000.** Natural and synthetic podolactones with potential use as natural herbicide models. *Journal of agricultural and food chemistry* **48**(7): 3003-3007.
247. **Madigan MT, Martinko JM, Parker J. 2017.** *Brock biology of microorganisms*: Pearson.
248. **Mallik A 1998.** Allelopathy and competition in coniferous forests. *Environmental Forest Science*: Springer, 309-315.
249. **Mangan SA, Schnitzer SA, Herre EA, Mack KM, Valencia MC, Sanchez EI, Bever JD. 2010.** Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* **466**(7307): 752-755.
250. **Marchand P, Comita LS, Wright SJ, Condit R, Hubbell SP, Beckman NG. 2019.** Seed-to-seedling transitions exhibit distance-dependent mortality but no strong spacing effects in a Neotropical forest. *Ecology*.
251. **Mariotte P, Mehrabi Z, Bezemer TM, De Deyn GB, Kulmatiski A, Drigo B, Veen GC, Van der Heijden MG, Kardol P. 2018.** Plant–soil feedback: bridging natural and agricultural sciences. *Trends in ecology & evolution* **33**(2): 129-142.
252. **Marks GC. 2012.** *Ectomycorrhizae: their ecology and physiology*: Elsevier.
253. **Marx D. 1969.** Modified Melin-Norkrans Medium. *Phytophathology* **59**: 152-163.
254. **Mathur S. 1970.** Degradation of soil humus by the fairy ring mushroom. *Plant and Soil* **33**(1-3): 717-720.
255. **Mayor JR, Henkel TW. 2006.** Do ectomycorrhizas alter leaf-litter decomposition in monodominant tropical forests of Guyana? *New Phytologist* **169**(3): 579-588.
256. **Mazzoleni S, Bonanomi G, Giannino F, Incerti G, Dekker SC, Rietkerk M. 2010.** Modelling the effects of litter decomposition on tree diversity patterns. *Ecological modelling* **221**(23): 2784-2792.
257. **Mazzoleni S, Bonanomi G, Giannino F, Rietkerk M, Dekker S, Zucconi F. 2007.** Is plant biodiversity driven by decomposition processes? An emerging new theory on plant diversity. *Community Ecology* **8**(1): 103-109.
258. **Mazzoleni S, Bonanomi G, Incerti G, Chiusano Maria L, Termolino P, Mingo A, Senatore M, Giannino F, Cartenì F, Rietkerk M, et al. 2014.** Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negative plant–soil feedbacks? *New Phytologist* **205**(3): 1195-1210.
259. **Mazzoleni S, Bonanomi G, Incerti G, Chiusano ML, Termolino P, Mingo A, Senatore M, Giannino F, Cartenì F, Rietkerk M. 2015a.** Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negative plant–soil feedbacks? *New Phytologist* **205**(3): 1195-1210.
260. **Mazzoleni S, Cartenì F, Bonanomi G, Senatore M, Termolino P, Giannino F, Incerti G, Rietkerk M, Lanzotti V, Chiusano Maria L. 2015b.** Inhibitory effects of extracellular self-DNA: a general biological process? *New Phytologist* **206**(1): 127-132.
261. **Mazzoleni S, Cartenì F, Bonanomi G, Senatore M, Termolino P, Giannino F, Incerti G, Rietkerk M, Lanzotti V, Chiusano ML. 2015c.** Inhibitory effects of extracellular self-DNA: a general biological process? *New Phytologist* **206**(1): 127-132.
262. **McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. 2012.** An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME journal* **6**(3): 610.

263. **McGuire KL. 2007.** Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest. *Ecology* **88**(3): 567-574.
264. **McGuire KL, Allison SD, Fierer N, Treseder KK. 2013.** Ectomycorrhizal-dominated boreal and tropical forests have distinct fungal communities, but analogous spatial patterns across soil horizons. *PLoS One* **8**(7): e68278.
265. **McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat I, Marchant R, Smyth W. 2001.** Microbial decolourisation and degradation of textile dyes. *Applied microbiology and biotechnology* **56**(1-2): 81-87.
266. **McNaughton S. 1968.** Autotoxic feedback in relatin to germination and seedling growth in Typha latifolia. *Ecology* **49**(2): 367-369.
267. **Meiners SJ. 2014.** Functional correlates of allelopathic potential in a successional plant community. *Plant ecology* **215**(6): 661-672.
268. **Melillo JM, Aber JD, Muratore JF. 1982.** Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* **63**(3): 621-626.
269. **Miller F, Harper E, Macauley B. 1989.** Field examination of temperature and oxygen relationships in mushroom composting stacks dash consideration of stack oxygenation based on utilisation and supply. *Australian Journal of Experimental Agriculture* **29**(5): 741-750.
270. **Miller G, Grand L, Tredway L. 2011.** Identification and distribution of fungi associated with fairy rings on golf putting greens. *Plant disease* **95**(9): 1131-1138.
271. **Miller G, Soika M, Tredway L. 2012.** Evaluation of preventive fungicide applications for fairy ring control in golf putting greens and in vitro sensitivity of fairy ring species to fungicides. *Plant disease* **96**(7): 1001-1007.
272. **Mills KE, Bever JD. 1998.** Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. *Ecology* **79**(5): 1595-1601.
273. **Mitchinson A. 2014.** Plant science: Fairy chemicals. *Nature* **505**(7483): 298.
274. **Mittermeier RA, Turner WR, Larsen FW, Brooks TM, Gascon C 2011.** Global biodiversity conservation: the critical role of hotspots. *Biodiversity hotspots*: Springer, 3-22.
275. **Mohan B, Hosetti B. 1999.** Aquatic plants for toxicity assessment. *Environmental research* **81**(4): 259-274.
276. **Molter DJ. 2019.** On mycorrhizal individuality. *Biology & Philosophy* **34**(5): 52.
277. **Moore D, Gange AC, Gange EG, Boddy L 2008**. Fruit bodies: their production and development in relation to environment. *British Mycological Society Symposia Series*: Elsevier. 79-103.
278. **Moorhead DL, Sinsabaugh RL. 2006.** A theoretical model of litter decay and microbial interaction. *Ecological Monographs* **76**(2): 151-174.
279. **Morath SU, Hung R, Bennett JW. 2012.** Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. *Fungal Biology Reviews* **26**(2-3): 73-83.
280. **Murata H, Ohta A, Yamada A, Narimatsu M, Futamura N. 2005.** Genetic mosaics in the massive persisting rhizosphere colony "shiro" of the ectomycorrhizal basidiomycete Tricholoma matsutake. *Mycorrhiza* **15**(7): 505-512.
281. **Narimatsu M, Koiwa T, Masaki T, Sakamoto Y, Ohmori H, Tawaraya K. 2015.** Relationship between climate, expansion rate, and fruiting in fairy rings ('shiro') of an ectomycorrhizal fungus Tricholoma matsutake in a Pinus densiflora forest. *Fungal Ecology* **15**: 18-28.
282. **Natsch A, Keel C, Pfirter HA, Haas D, Défago G. 1994.** Contribution of the global regulator gene gacA to persistence and dissemination of Pseudomonas fluorescens biocontrol strain CHA0 introduced into soil microcosms. *Applied and environmental microbiology* **60**(7): 2553-2560.
283. **Nelson SC. 2008.** *Fairy Rings: Toadstools, Stinkhorns, and Puffballs*: Honolulu, Hawai'i.
284. **Nesme J, Achouak W, Agathos SN, Bailey M, Baldrian P, Brunel D, Frostegård Å, Heulin T, Jansson JK, Jurkevitch E. 2016.** Back to the future of soil metagenomics. *Frontiers in microbiology* **7**: 73.
285. **Newell K. 1984.** Interaction between two decomposer basidiomycetes and a collembolan under Sitka spruce: grazing and its potential effects on fungal distribution and litter decomposition. *Soil Biology and Biochemistry* **16**(3): 235-239.

286. **Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016.** FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241-248.
287. **Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L. 2018.** The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic acids research* **47**(D1): D259-D264.
288. **Nishino K. 2017.** Chemical ecology of the (oxalato) aluminate complex as an antimicrobial substance from the "shiro" of Tricholoma matsutake.
289. **Nishino K, Shiro M, Okura R, Oizumi K, Fujita T, Sasamori T, Tokitoh N, Yamada A, Tanaka C, Yamaguchi M. 2017.** The (oxalato) aluminate complex as an antimicrobial substance protecting the "shiro" of Tricholoma matsutake from soil micro-organisms. *Bioscience, biotechnology, and biochemistry* **81**(1): 102-111.
290. **Norstadt FA, Frey C, Sigg H. 1973.** Soil Urease: Paucity in the Presence of the Fairy Ring Fungus Marasmius oreades (Bolt.) Fr. 1. *Soil Science Society of America Journal* **37**(6): 880-885.
291. **Oh S-Y, Fong JJ, Park MS, Lim YW. 2016.** Distinctive feature of microbial communities and bacterial functional profiles in Tricholoma matsutake dominant soil. *PloS one* **11**(12): e0168573.
292. **Oh S-Y, Lim YW. 2018a.** Effect of fairy ring bacteria on the growth of Tricholoma matsutake in vitro culture. *Mycorrhiza* **28**(5-6): 411-419.
293. **Oh S-Y, Lim YW. 2018b.** Root-associated bacteria influencing mycelial growth of Tricholoma matsutake (pine mushroom). *Journal of Microbiology* **56**(6): 399-407.
294. **Oh S-Y, Park MS, Cho HJ, Lim YW. 2018.** Diversity and effect of Trichoderma isolated from the roots of Pinus densiflora within the fairy ring of pine mushroom (Tricholoma matsutake). *PloS one* **13**(11): e0205900.
295. **Ohara H, Hamada M. 1967.** Disappearance of bacteria from the zone of active mycorrhizas in Tricholoma matsutake (S. Ito et Imai) Singer. *Nature* **213**(5075): 528-529.
296. **Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GV, Underwood EC, D'amico JA, Itoua I, Strand HE, Morrison JC. 2001.** Terrestrial Ecoregions of the World: A New Map of Life on EarthA new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience* **51**(11): 933-938.
297. **Osono T. 2002.** Phyllosphere fungi on leaf litter of Fagus crenata: occurrence, colonization, and succession. *Canadian Journal of Botany* **80**(5): 460-469.
298. **Osono T. 2007.** Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecological Research* **22**(6): 955-974.
299. **Osono T, Takeda H. 2001a.** Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-year decomposition processes in a cool temperate deciduous forest in Japan. *Ecological Research* **16**(4): 649-670.
300. **Osono T, Takeda H. 2001b.** Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-year decomposition processes in a cool temperate deciduous forest in Japan. *Ecological Research* **16**(4): 649-670.
301. **Packer A, Clay K. 2000.** Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature* **404**(6775): 278.
302. **Pansu M, Thuriès L. 2003.** Kinetics of C and N mineralization, N immobilization and N volatilization of organic inputs in soil. *Soil Biology and Biochemistry* **35**(1): 37-48.
303. **Park DS, Peterson C, Zhao S, Coats JR. 2004.** Fumigation toxicity of volatile natural and synthetic cyanohydrins to stored-product pests and activity as soil fumigants. *Pest Management Science: formerly Pesticide Science* **60**(8): 833-838.
304. **Park MS, Oh S-Y, Cho HJ, Fong JJ, Cheon W-J, Lim YW. 2014.** Trichoderma songyi sp. nov., a new species associated with the pine mushroom (Tricholoma matsutake). *Antonie Van Leeuwenhoek* **106**(4): 593-603.
305. **Parke JL, Gurian-Sherman D. 2001.** Diversity of the Burkholderia cepacia complex and implications for risk assessment of biological control strains. *Annual review of phytopathology* **39**(1): 225-258.
306. **Parker-Rhodes A. 1955.** Fairy ring kinetics. *Transactions of the British Mycological Society* **38**(1): 59-72.

307. **Pärtel M, Bruun HH, Sammul M. 2005.** Biodiversity in temperate European grasslands: origin and conservation. *Grassland science in Europe* **10**(1): 14.
308. **Patrick Z. 1971.** Phytotoxic substances associated with the decomposition in soil of plant residues. *Soil Science* **111**(1): 13-18.
309. **Paul EA. 2014.** *Soil microbiology, ecology and biochemistry*: Academic press.
310. **Pavoine S, Vela E, Gachet S, De Bélair G, Bonsall MB. 2011.** Linking patterns in phylogeny, traits, abiotic variables and space: a novel approach to linking environmental filtering and plant community assembly. *Journal of Ecology* **99**(1): 165-175.
311. **Peh KS-H, Sonké B, Séné O, Djuikouo M-NK, Nguembou CK, Taedoumg H, Begne SK, Lewis SL. 2014.** Mixed-forest species establishment in a monodominant forest in central Africa: implications for tropical forest invasibility. *PLoS One* **9**(5): e97585.
312. **Peh KSH, Lewis SL, Lloyd J. 2011.** Mechanisms of monodominance in diverse tropical tree-dominated systems. *Journal of Ecology* **99**(4): 891-898.
313. **Peralta-Maraver I, López-Rodríguez MJ, Fenoglio S, Bo T, Luzón-Ortega JM, de Figueroa JMT. 2011.** Macroinvertebrate colonization of two different tree species leaf packs (native vs. introduced) in a Mediterranean stream. *Journal of Freshwater Ecology* **26**(4): 495-505.
314. **Perez-Moreno J, Read D. 2000.** Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. *The New Phytologist* **145**(2): 301-309.
315. **Petermann JS, Fergus AJ, Turnbull LA, Schmid B. 2008.** Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* **89**(9): 2399-2406.
316. **Philpott CC. 2006.** Iron uptake in fungi: a system for every source. *Biochimica et Biophysica Acta (bba)-molecular cell research* **1763**(7): 636-645.
317. **Pleban S, Chernin L, Chet I. 1997.** Chitinolytic activity of an endophytic strain of Bacillus cereus. *Letters in Applied Microbiology* **25**(4): 284-288.
318. **Powell K 1993.** The commercial exploitation of microorganisms in agriculture. *Exploitation of microorganisms*: Springer, 441-459.
319. **Preston CM, Nault JR, Trofymow J. 2009a.** Chemical changes during 6 years of decomposition of 11 litters in some Canadian forest sites. Part 2. 13 C abundance, solid-state 13 C NMR spectroscopy and the meaning of "lignin". *Ecosystems* **12**(7): 1078-1102.
320. **Preston CM, Nault JR, Trofymow J. 2009b.** Chemical changes during 6 years of decomposition of 11 litters in some Canadian forest sites. Part 2. 13C abundance, solid-state 13C NMR spectroscopy and the meaning of "lignin". *Ecosystems* **12**(7): 1078-1102.
321. **Pugh G. 1980.** Strategies in fungal ecology. *Transactions of the British Mycological Society* **75**(1): 1-14.
322. **Pukkala T, Möykkynen T, Thor M, Rönnberg J, Stenlid J. 2005.** Modeling infection and spread of Heterobasidion annosum in even-aged Fennoscandian conifer stands. *Canadian Journal of Forest Research* **35**(1): 74-84.
323. **Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F. 2016.** Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular Ecology* **25**(16): 4059-4074.
324. **Putnam AR, DeFrank J, Barnes JP. 1983.** Exploitation of allelopathy for weed control in annual and perennial cropping systems. *Journal of Chemical Ecology* **9**(8): 1001-1010.
325. **Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2012.** The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research* **41**(D1): D590-D596.
326. **Quézel P, Médail F. 2017.** Ecologie et biogéographie des forêts du bassin méditerranéen.
327. **Read D, Perez-Moreno J. 2003.** Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance? *New Phytologist* **157**(3): 475-492.
328. **Read DJ. 1991.** Mycorrhizas in ecosystems. *Experientia* **47**(4): 376-391.
329. **Read J, Hallam P, Cherrier J-F. 1995.** The anomaly of monodominant tropical rainforests: some preliminary observations in the Nothofagus-dominated rainforests of New Caledonia. *Journal of Tropical Ecology* **11**(3): 359-389.
330. **Rice EL. 2012.** *Allelopathy*: Academic press.
331. **Rice EL. 2013.** *Allelopathy*: Academic Press.

332. **Richards PW. 1952.** *The tropical rain forest; an ecological study*: At The University Press; Cambridge.
333. **Rillig MC. 2005.** A connection between fungal hydrophobins and soil water repellency? *Pedobiologia* **49**(5): 395-399.
334. **Rinkes ZL, Weintraub MN, DeForest JL, Moorhead DL. 2011.** Microbial substrate preference and community dynamics during decomposition of Acer saccharum. *Fungal Ecology* **4**(6): 396-407.
335. **Ritz K. 1995.** Growth responses of some soil fungi to spatially heterogeneous nutrients. *FEMS Microbiology Ecology* **16**(4): 269-279.
336. **Rodrigues ML, Godinho RM, Zamith-Miranda D, Nimrichter L. 2015.** Traveling into outer space: unanswered questions about fungal extracellular vesicles. *PLoS pathogens* **11**(12): e1005240.
337. **Rønn R, McCaig AE, Griffiths BS, Prosser JI. 2002.** Impact of protozoan grazing on bacterial community structure in soil microcosms. *Applied and environmental microbiology* **68**(12): 6094-6105.
338. **Rose SJ, Burnside OC, Specht JE, Swisher BA. 1984.** Competition and Allelopathy Between Soybeans and Weeds1. *Agronomy Journal* **76**(4): 523-528.
339. **Rosenberg K, Bertaux J, Krome K, Hartmann A, Scheu S, Bonkowski M. 2009.** Soil amoebae rapidly change bacterial community composition in the rhizosphere of Arabidopsis thaliana. *The ISME journal* **3**(6): 675.
340. **Rosling A, Landeweert R, Lindahl B, Larsson KH, Kuyper T, Taylor A, Finlay R. 2003.** Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New Phytologist* **159**(3): 775-783.
341. **Ruinen J. 1956.** Occurrence of Beijerinckia species in the 'phyllosphere'. *Nature* **177**(4501): 220.
342. **Rütting T, Boeckx P, Müller C, Klemedtsson L. 2011.** Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* **8**(7): 1779-1791.
343. **Rytioja J, Hildén K, Yuzon J, Hatakka A, de Vries RP, Mäkelä MR. 2014.** Plant-polysaccharide-degrading enzymes from basidiomycetes. *Microbiol. Mol. Biol. Rev.* **78**(4): 614-649.
344. **Sagara N. 1975.** Ammonia fungi: a chemoecological grouping of terrestrial fungi.
345. **Santelli CM, Webb SM, Dohnalkova AC, Hansel CM. 2011.** Diversity of Mn oxides produced by Mn (II)-oxidizing fungi. *Geochimica et Cosmochimica Acta* **75**(10): 2762-2776.
346. **Sardans J, Peñuelas J. 2005.** Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. *Soil Biology and Biochemistry* **37**(3): 455-461.
347. **Sarker TC, Incerti G, Spaccini R, Piccolo A, Mazzoleni S, Bonanomi G. 2018.** Linking organic matter chemistry with soil aggregate stability: Insight from 13 C NMR spectroscopy. *Soil Biology and Biochemistry* **117**: 175-184.
348. **Scarpa FM, Valio I. 2008.** Relationship between seed size and litter effects on early seedling establishment of 15 tropical tree species. *Journal of Tropical Ecology* **24**(5): 569-573.
349. **Schimel JP, Gulledge JM, Clein-Curley JS, Lindstrom JE, Braddock JF. 1999.** Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. *Soil Biology and Biochemistry* **31**(6): 831-838.
350. **Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K. 2012.** Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *The ISME journal* **6**(9): 1749.
351. **Schrijver AD, Mot RD. 1999.** Degradation of pesticides by actinomycetes. *Critical reviews in microbiology* **25**(2): 85-119.
352. **Schupp EW. 1992.** The Janzen-Connell model for tropical tree diversity: population implications and the importance of spatial scale. *The American Naturalist* **140**(3): 526-530.
353. **Schupp EW, Jordano P. 2011.** The full path of Janzen–Connell effects: genetic tracking of seeds to adult plant recruitment. *Molecular ecology* **20**(19): 3953-3955.
354. **Semchenko M, Leff JW, Lozano YM, Saar S, Davison J, Wilkinson A, Jackson BG, Pritchard WJ, Jonathan R, Oakley S. 2018.** Fungal diversity regulates plant-soil feedbacks in temperate grassland. *Science advances* **4**(11): eaau4578.
355. **Shantz HL, Piemeisel R. 1917.** *Fungus fairy rings in eastern Colorado and their effects on vegetation*: US Government Printing Office.
356. **Shaw III C. 1980.** Characteristics of Armillaria mellea on pine (Pinus ponderosa) root systems in expanding centers of root rot. *Northwest Science* **54**(2): 137-145.

357.**Sietsma J, Wösten H, Wessels J. 1995.** Cell wall growth and protein secretion in fungi. *Canadian Journal of Botany* **73**(S1): 388-395.
358.**Silvertown J, Law R. 1987.** Do plants need niches? Some recent developments in plant community ecology. *Trends in ecology & evolution* **2**(1): 24-26.
359.**Simon J-C, Marchesi JR, Mougel C, Selosse M-A. 2019.** Host-microbiota interactions: from holobiont theory to analysis. *Microbiome* **7**(1): 5.
360.**Singh H, Batish DR, Kohli R. 1999.** Autotoxicity: concept, organisms, and ecological significance. *Critical Reviews in Plant Sciences* **18**(6): 757-772.
361.**Singh PP, Shin YC, Park CS, Chung YR. 1999.** Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. *Phytopathology* **89**(1): 92-99.
362.**Smith-Ramesh LM, Reynolds HL. 2017.** The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. *Journal of vegetation Science* **28**(3): 484-494.
363.**Smith J. 1980.** Is biologic control of Marasmius oreades fairy rings possible? *Plant Disease* **64**(4): 348-354.
364.**Smith SE, Read DJ. 2010.** *Mycorrhizal symbiosis*: Academic press.
365.**Smoot ME, Ono K, Ruscheinski J, Wang P-L, Ideker T. 2010.** Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* **27**(3): 431-432.
366.**Šnajdr J, Dobiášová P, Vetrovský T, Valášková V, Alawi A, Boddy L, Baldrian P. 2011.** Saprotrophic basidiomycete mycelia and their interspecific interactions affect the spatial distribution of extracellular enzymes in soil. *FEMS Microbiology Ecology* **78**(1): 80-90.
367.**Snedaker SC 1982.** Mangrove species zonation: why? *Contributions to the Ecology of Halophytes*: Springer, 111-125.
368.**Spiteller P. 2008.** Chemical defence strategies of higher fungi. *Chemistry–A European Journal* **14**(30): 9100-9110.
369.**Splivallo R, Novero M, Bertea CM, Bossi S, Bonfante P. 2007.** Truffle volatiles inhibit growth and induce an oxidative burst in Arabidopsis thaliana. *New Phytologist* **175**(3): 417-424.
370.**Stevenson DR, Thompson CJ. 1976.** Fairy ring kinetics. *Journal of theoretical biology* **58**(1): 143-163.
371.**Steyn P, Segers P, Vancanneyt M, Sandra P, Kersters K, Joubert J. 1998.** Classification of heparinolytic bacteria into a new genus, Pedobacter, comprising four species: Pedobacter heparinus comb. nov., Pedobacter piscium comb. nov., Pedobacter africanus sp. nov. and Pedobacter saltans sp. nov. proposal of the family Sphingobacteriaceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology* **48**(1): 165-177.
372.**Sumorok B, Kosiński K, Michalska-Hejduk D, Kiedrzyńska E. 2008.** Distribution of ectomycorrhizal fungi in periodically inundated plant communities on the Pilica River floodplain. *Ecohydrology & Hydrobiology* **8**(2): 401-410.
373.**Sutton B. 1990.** Fungal Diseases of Amenity Turf Grasses, JD Smith, N. Jackson, AR Woolhouse (Eds.), E. & FN Spon, London & New York (1989), p. 401, Price£ 78.50. *Mycological Research* **94**(3): 432.
374.**Suzuki T, Yamamoto N, Choi J-H, Takano T, Sasaki Y, Terashima Y, Ito A, Dohra H, Hirai H, Nakamura Y. 2016.** The biosynthetic pathway of 2-azahypoxanthine in fairy-ring forming fungus. *Scientific reports* **6**: 39087.
375.**Swift MJ, Heal OW, Anderson JM, Anderson J. 1979.** *Decomposition in terrestrial ecosystems*: Univ of California Press.
376.**Tagliavini M, Marangoni B. 1992.** Growth of peach as affected by decomposition of own root residues in soil. *Plant and Soil* **145**(2): 253-260.
377.**Takano T, Yamamoto N, Suzuki T, Dohra H, Choi J-H, Terashima Y, Yokoyama K, Kawagishi H, Yano K. 2019.** Genome sequence analysis of the fairy ring-forming fungus Lepista sordida and gene candidates for interaction with plants. *Scientific reports* **9**(1): 5888.
378.**Takasaki K, Shoun H, Yamaguchi M, Takeo K, Nakamura A, Hoshino T, Takaya N. 2004.** Fungal Ammonia Fermentation, a Novel Metabolic Mechanism That Couples the Dissimilatory and Assimilatory Pathways of Both Nitrate and Ethanol ROLE OF ACETYL CoA SYNTHETASE IN ANAEROBIC ATP SYNTHESIS. *Journal of Biological Chemistry* **279**(13): 12414-12420.
379.**Talbot J, Treseder K. 2010.** Controls over mycorrhizal uptake of organic nitrogen. *Pedobiologia* **53**(3): 169-179.

380. **Talbot JM, Bruns TD, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Peay KG. 2013.** Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. *Soil Biology and Biochemistry* **57**: 282-291.
381. **Taylor BR, Parkinson D, Parsons WF. 1989.** Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology* **70**(1): 97-104.
382. **Ter Steege H, Pitman N, Sabatier D, Castellanos H, Van Der Hout P, Daly DC, Silveira M, Phillips O, Vasquez R, Van Andel T. 2003.** A spatial model of tree α-diversity and tree density for the Amazon. *Biodiversity & Conservation* **12**(11): 2255-2277.
383. **Terashima Y, Fukiharu T, Fujiie A. 2004.** Morphology and comparative ecology of the fairy ring fungi, Vascellum curtisii and Bovista dermoxantha, on turf of bentgrass, bluegrass, and Zoysiagrass. *Mycoscience* **45**(4): 251-260.
384. **Teste FP, Kardol P, Turner BL, Wardle DA, Zemunik G, Renton M, Laliberté E. 2017.** Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science* **355**(6321): 173-176.
385. **Thomashow LS, LeTourneau MK, Kwak YS, Weller DM. 2019.** The soil-borne legacy in the age of the holobiont. *Microbial biotechnology* **12**(1): 51-54.
386. **Tilman D, Downing JA. 1994.** Biodiversity and stability in grasslands. *Nature* **367**(6461): 363.
387. **Tláskal V, Voříšková J, Baldrian P. 2016.** Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS microbiology ecology* **92**(11): fiw177.
388. **Tomasi G, Scandola M, Briese BH, Jendrossek D. 1996.** Enzymatic degradation of bacterial poly (3-hydroxybutyrate) by a depolymerase from Pseudomonas lemoignei. *Macromolecules* **29**(2): 507-513.
389. **Torti SD, Coley PD, Kursar TA. 2001.** Causes and consequences of monodominance in tropical lowland forests. *The American Naturalist* **157**(2): 141-153.
390. **Trejo-Estrada S, Paszczynski A, Crawford D. 1998.** Antibiotics and enzymes produced by the biocontrol agent Streptomyces violaceusniger YCED-9. *Journal of Industrial Microbiology and Biotechnology* **21**(1-2): 81-90.
391. **Tribe HT, Weber RW. 2002.** A low-temperature fungus from cardboard, Myxotrichum chartarum. *Mycologist* **16**(1): 3-5.
392. **Tuomi J, Kytöviita MM, Härdling R. 2001.** Cost efficiency of nutrient acquisition and the advantage of mycorrhizal symbiosis for the host plant. *Oikos* **92**(1): 62-70.
393. **Turner BL, Cernusak LA. 2011.** Ecology of the Podocarpaceae in tropical forests.
394. **Urbanová M, Šnajdr J, Baldrian P. 2015.** Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry* **84**: 53-64.
395. **Valencia R, Balslev H, Miño GPY. 1994.** High tree alpha-diversity in Amazonian Ecuador. *Biodiversity & Conservation* **3**(1): 21-28.
396. **VAN ANDEL J, Bakker J, Grootjans A. 1993.** Mechanisms of vegetation succession: a review of concepts and perspectives. *Acta botanica neerlandica* **42**(4): 413-433.
397. **van de Voorde TF, van der Putten WH, Martijn Bezemer T. 2011.** Intra-and interspecific plant–soil interactions, soil legacies and priority effects during old-field succession. *Journal of Ecology* **99**(4): 945-953.
398. **Van Der Heijden MG, Bardgett RD, Van Straalen NM. 2008.** The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters* **11**(3): 296-310.
399. **Van der Heijden MG, Boller T, Wiemken A, Sanders IR. 1998.** Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* **79**(6): 2082-2091.
400. **Van der Heijden MG, Sanders IR 2002.** Mycorrhizal ecology: synthesis and perspectives. *Mycorrhizal ecology*: Springer, 441-456.
401. **van der Maarel E, Sykes MT. 1993.** Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. *Journal of vegetation Science* **4**(2): 179-188.
402. **Van der Putten W, Peters B, Van den Berg M. 1997.** Effects of litter on substrate conditions and growth of emergent macrophytes. *The New Phytologist* **135**(3): 527-537.

403. **van der Putten WH. 1997.** Die-back of Phragmites australis in European wetlands: an overview of the European research programme on reed die-back and progression (1993–1994). *Aquatic botany* **59**(3-4): 263-275.
404. **Van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA. 2013.** Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology* **101**(2): 265-276.
405. **Vazquez E, Amalfitano S, Fazi S, Butturini A. 2011.** Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions. *Biogeochemistry* **102**(1-3): 59-72.
406. **Veen C, Fry E, ten Hooven F, Kardol P, Morriën E, De Long JR. 2019.** The role of plant litter in driving plant-soil feedbacks. *Frontiers in Environmental Science* **7**: 168.
407. **Villela DM, Proctor J. 2002.** Leaf Litter Decomposition and Monodominance in the Peltogyne Forest of Maracá Island, Brazil1. *Biotropica* **34**(3): 334-347.
408. **Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M. 2008.** Trichoderma–plant–pathogen interactions. *Soil Biology and Biochemistry* **40**(1): 1-10.
409. **Vincenot CE, Cartenì F, Bonanomi G, Mazzoleni S, Giannino F. 2017.** Plant–soil negative feedback explains vegetation dynamics and patterns at multiple scales. *Oikos* **126**(9): 1319-1328.
410. **Voříšková J, Baldrian P. 2013.** Fungal community on decomposing leaf litter undergoes rapid successional changes. *The ISME journal* **7**(3): 477.
411. **Wallenstein MD, Haddix ML, Ayres E, Steltzer H, Magrini-Bair KA, Paul EA. 2013.** Litter chemistry changes more rapidly when decomposed at home but converges during decomposition–transformation. *Soil Biology and Biochemistry* **57**: 311-319.
412. **WANG Q, JIANG W, CHEN B. 2005.** Effects of fairy ring growth of Armillaria luteo-virens on soil fertility and plant community [J]. *Chinese Journal of Ecology* **3**.
413. **Warcup J. 1959.** Studies on Basidiomycetes in soil. *Transactions of the British Mycological Society* **42**(1): 45-52.
414. **Watt AS. 1947.** Pattern and process in the plant community. *Journal of Ecology* **35**(1/2): 1-22.
415. **Weston LA. 2000.** Are laboratory bioassays for allelopathy suitable for prediction of field responses? *Journal of Chemical Ecology* **26**(9): 2111-2118.
416. **Whitmore T. 1978.** Gaps in the forest canopy. *Tropical trees as living systems.*: 639-655.
417. **Wickings K, Grandy AS, Reed SC, Cleveland CC. 2012.** The origin of litter chemical complexity during decomposition. *Ecology letters* **15**(10): 1180-1188.
418. **Wilkinson DM. 1999.** The disturbing history of intermediate disturbance. *Oikos*: 145-147.
419. **Williamson JD, Quatrano RS. 1988.** ABA-regulation of two classes of embryo-specific sequences in mature wheat embryos. *Plant physiology* **86**(1): 208-215.
420. **Wilson JB, Peet RK, Dengler J, Pärtel M. 2012.** Plant species richness: the world records. *Journal of vegetation Science* **23**(4): 796-802.
421. **Wollaston WH. 1807.** VII. On Fairy-rings. *Philosophical Transactions of the Royal Society of London* **97**: 133-138.
422. **Woolley LP, Henkel TW, Sillett SC. 2008.** Reiteration in the monodominant tropical tree Dicymbe corymbosa (Caesalpiniaceae) and its potential adaptive significance. *Biotropica* **40**(1): 32-43.
423. **Wright JS. 2002.** Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* **130**(1): 1-14.
424. **Wright SJ. 1992.** Seasonal drought, soil fertility and the species density of tropical forest plant communities. *Trends in ecology & evolution* **7**(8): 260-263.
425. **Wurst S, Kaiser N, Nitzsche S, Haase J, Auge H, Rillig MC, Powell JR. 2015.** Tree diversity modifies distance-dependent effects on seedling emergence but not plant–soil feedbacks of temperate trees. *Ecology* **96**(6): 1529-1539.
426. **Xing R, Yan Hy, Gao Qb, Zhang Fq, Wang Jl, Chen Sl. 2018.** Microbial communities inhabiting the fairy ring of Floccularia luteovirens and isolation of potential mycorrhiza helper bacteria. *Journal of basic microbiology* **58**(6): 554-563.
427. **Xiong S, Nilsson C. 1999.** The effects of plant litter on vegetation: a meta-analysis. *Journal of Ecology* **87**(6): 984-994.

428.**Xu X, Ouyang H, Cao G, Richter A, Wanek W, Kuzyakov Y. 2011.** Dominant plant species shift their nitrogen uptake patterns in response to nutrient enrichment caused by a fungal fairy in an alpine meadow. *Plant and soil* **341**(1-2): 495-504.
429.**Yang C, Li J, Liu N, Zhang Y. 2019.** Effects of fairy ring fungi on plants and soil in the alpine and temperate grasslands of China. *Plant and Soil*: 1-12.
430.**Yang C, Li J, Zhang F, Liu N, Zhang Y. 2018a.** The optimal Redfield N: P ratio caused by fairy ring fungi stimulates plant productivity in the temperate steppe of China. *Fungal ecology* **34**: 91-98.
431.**Yang C, Zhang F, Liu N, Hu J, Zhang Y. 2018b.** Changes in soil bacterial communities in response to the fairy ring fungus Agaricus gennadii in the temperate steppes of China. *Pedobiologia* **69**: 34-40.
432.**Yang C, Zhang Y, Rong Y, Bei Y, Wei Y, Liu N. 2018c.** Temporal variation of Q10 values in response to changes in soil physiochemical properties caused by fairy rings. *European journal of soil biology* **86**: 42-48.
433.**Yu S-M, Lee YH. 2013.** Plant growth promoting rhizobacterium Proteus vulgaris JBLS202 stimulates the seedling growth of Chinese cabbage through indole emission. *Plant and Soil* **370**(1-2): 485-495.
434.**Yun W, Hall IR. 2004.** Edible ectomycorrhizal mushrooms: challenges and achievements. *Canadian Journal of Botany* **82**(8): 1063-1073.
435.**ZengPu L, JunRan J, Changwen W. 1994.** Antagonism between Ectomycorrhizal fungi and plant pathogens. *Mycorrhizas for Plantation Forestry in Asia. Brundett M, Dell B, Malajczuk N, and Mingqin G.(eds)*: 77-81.
436.**Zimmerman JK, Pulliam WM, Lodge DJ, Quinones-Orfila V, Fetcher N, Guzman-Grajales S, Parrotta JA, Asbury CE, Walker LR, Waide RB. 1995.** Nitrogen immobilization by decomposing woody debris and the recovery of tropical wet forest from hurricane damage. *Oikos*: 314-322.
437.**Zucconi F. 2003.** *Declino del suolo e stanchezza del terreno*: Pitagora.

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