UNIVERSITY OF NAPLES FEDERICO II

Ph.D. Course in APPLIED BIOLOGY
Curriculum MICROBIOLOGY-ECOLOGY

THE BEECH, FAGUS SYLVATICA *L. (FAGACEAE) IN THE ITALIAN APENNINES: AN ECOLOGY APPROACH TO UNDERSTAND ORGANIC MATTER QUANTITY AND QUALITY IN LEAF LITTER AND SOIL*

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Doctor Europaeus

This Ph.D. thesis has been considered eligible for the label *Doctor Europaeus* according to the criteria of the European University Association.

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1 Introduction

Beech forest in the Nebrodi Mountains (Sicily, Italy) during summer. The beech reaches the southernmost tip of its distribution range in the nearby slopes of the Etna volcano.

1.1 Global changes following human impact: an overview with focus on C cycle and forest ecosystems

Forests cover about 30% of the Earth's surface and they play several ecosystem services (e.g. wood and fuel production, soil and water conservation, home of biological diversity etc.) and their importance has been recognized for a very long time. In addition, quite recently, their major role in the global carbon cycle has been acknowledged. As a matter of fact, by exchanging carbon with the atmosphere through photosynthesis and respiration, they sequestrate a very high C amount in their biomass, and in soil organic matter (Fig. 1-1).

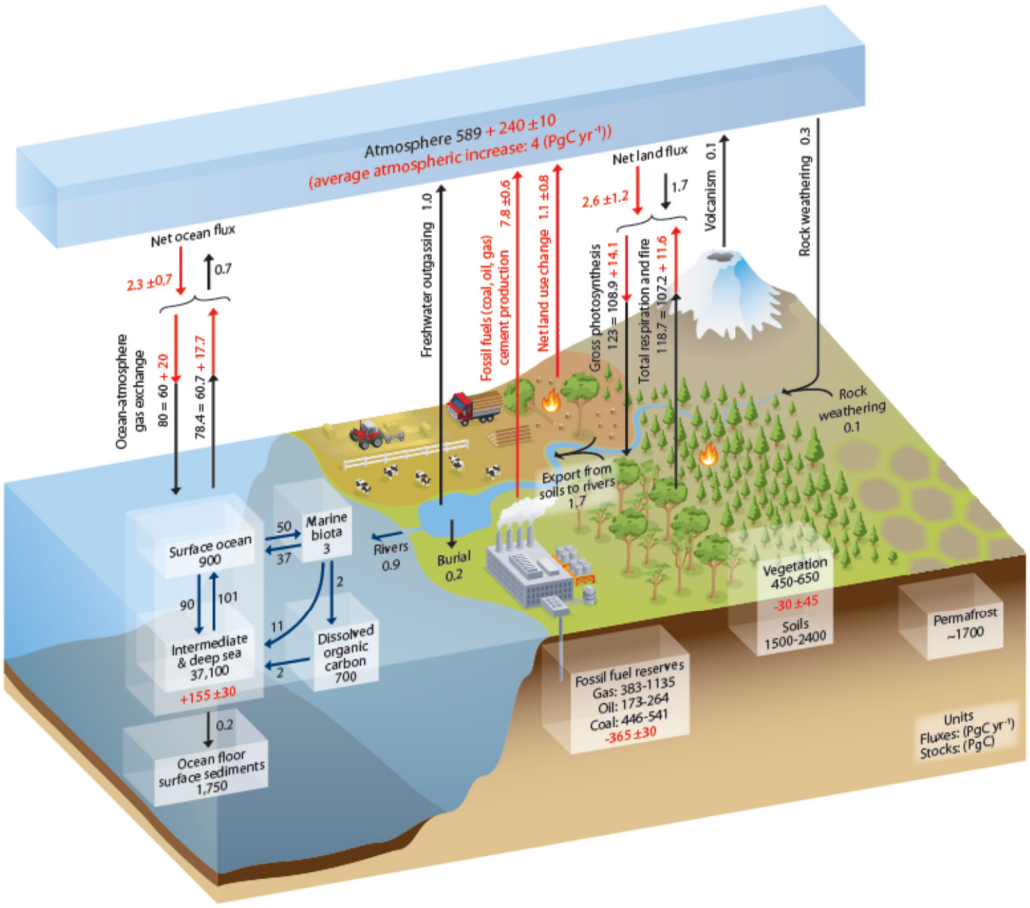


Figure 1-1: Simplified schematic of the global carbon cycle. Numbers represent reservoir mass, also called 'carbon stocks' in PgC (1 PgC = 1015 gC) and annual carbon exchange fluxes (in PgC yr^{-1}). Black numbers and arrows indicate reservoir mass and exchange fluxes estimated for the time prior to the Industrial Era, about 1750. Red numbers in the reservoirs denote cumulative changes of anthropogenic carbon over the Industrial Period 1750–2011. Adapted from IPCC (2013).

The recognition of this role is crucial not only in terms of knowledge and human economy (Scarfò and Mercurio, 2009), but also for the management of ecosystems. Natural or human-caused disturbances (e.g. fires, deforestation, change of land use etc.) cause a great release of CO_2 to the atmosphere. In contrast, several experimental and empirical studies have shown that proper use and management of forests could mitigate emissions of carbon dioxide. It has been demonstrated that

reforestation and afforestation, as well as vegetation regrowth after disturbance, cause a net absorption of CO_2 from the atmosphere. Therefore, the importance of this last aspect is evident in view of the ongoing global climatic changes (Dixon et al., 1994; Masera et al., 2003; Thuille et al., 2000).

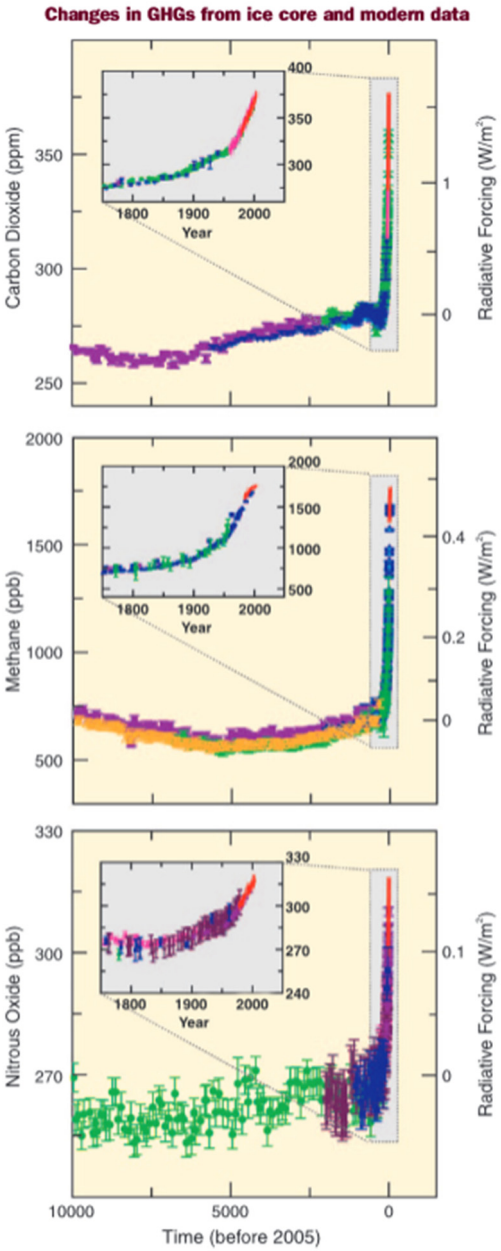


Figure 1-2: Atmospheric concentrations of CO_2, CH_4 and N_2O over the last 10.000 years (large panels) and since 1750(inset panels). Adapted from IPCC (2013)

The global climate system is undergoing changes over the last few hundred years which are, on average, sharp and unprecedented compared to the past millennia. During its evolutionary history, the earth witnessed concentration of atmospheric CO_2 fluctuating many times. Being CO_2 one of the most important greenhouse gas, the planet has undergone recurring temperature changes that have led to alternating periods of glaciation and inter-glaciation, resulting in change in the physiognomy of the land. Nevertheless, it is undeniable that the changes in recent times are surprising because of their rapidity and, likely, the main cause of this phenomenon is human activities.

The main greenhouse gases are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) whose atmospheric concentrations in 2011 were 391 ppm, 1803 ppb, and 324 ppb, respectively. These concentrations exceeded the pre-industrial levels by about 40%, 150%, and 20%, correspondingly (IPCC, 2013) (Fig. 1-2). These gases, absorbing the outgoing infrared radiations irradiated from the earth's surface, trap heat causing an increase of atmospheric temperature. An increase in the atmospheric temperature can cause serious damage to the land ecosystems (e.g. altered balance between photosynthesis and respiration in plants, different response of C3, C4 and CAM plants, change in land use, biodiversity reduction etc.) and marine ecosystems (e.g. ocean acidification, reduction or destructions of coral reefs, biodiversity loss etc.). Moreover, a temperature increase can affect the surface waters of the oceans with serious consequences for the increase in sea level, affecting human populations in a most dramatic way because most of the metropolis were raised at sea level and/or in

close proximity of it and, therefore, they would risk to be submerged. The analysis of climatic data also indicate a change in the amount and distribution of precipitation.

Forests can influence global warming in different ways such as, for example, the production of greenhouse gases such as carbon monoxide, ozone and nitrous oxides (Jandl et al., 2007) or the changing in albedo or reflectivity when the forests are converted to other land cover types. However, the forests influence climate change mainly through their CO_2 exchange with the atmosphere, widely discussed below.

In agreement with the estimates reported in the Global Forest Resources Assessment Report (FAO, 2010), forests cover about 4 billion hectares, that is 31% of total land. Most of them (52%) are in the tropical zones at low latitudes (0° to 25° N and S). At the latitudes between 50° to 75° N and S the area covered by forests is about 30% while at mid latitudes (25° to 50° N and S) it is about 18%. Unfortunately, during a fairly recent past, deforestation, particularly in the tropical regions has resulted in a considerable reduction of the forested areas (estimated to be about 15.4 million ha/y during the period 1980-1990).

On the contrary, the awareness of the importance of forests in mitigating CO_2 emissions and practices of reforestation and afforestation required by the Kyoto protocol has prompted several countries in temperate and boreal zone to reverse the tendency and recover the areas damaged in the past (Fig. 1-3).

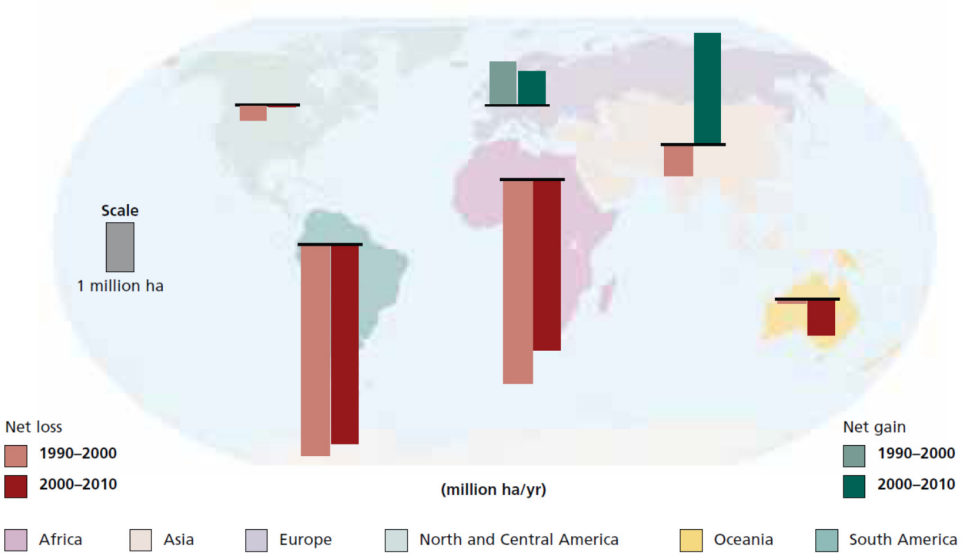


Figure 1-3: Annual change in forest area by region in the years 1990-2010. Adapted from FAO (2010).

An approximate estimate suggests that the world's forests store more than 650 billion tonnes of carbon, 44% in the living plant biomass above and below ground, 11% in dead plant biomass and in the organic horizon on the forest floor, and 45% in the mineral soil (FAO, 2010). Approximation of the estimates is especially due to the difficulty in measuring and often to different measurement methods. Thus, on the one hand, despite the variability of the methods adopted, the aboveground

biomass can be estimated with a good approximation. On the other hand, it is very difficult to assess the underground biomass (roots). It is known that, on average, only 50% of the roots is located above 20 cm and 75% above 40 cm (Jackson et al., 1996). It can be deduced that 25% of the roots go deeper and they are an important input of C in deeper soil horizons, yet even more unquantifiable. However, it is possible that the high levels of uncertainty are due to incomplete counts (Houghton, 2003).

When considering the distribution of C pools between the vegetation and the soil, it should be emphasized that there is a distinct difference between the types of forests, difference that is a function of latitude. As a matter of fact, tropical forests have 62% of carbon pool stored within the living biomass of vegetation; on the contrary, soils of temperate forests contain about 66% of total C pool and those of boreal forests about 75% (Dixon et al., 1994; Valentini et al., 2000).

Accumulation of soil C depends on balance between plant production and plant residue mineralization, hence between two important ecosystem functional processes such as primary net productivity and decomposition.

The primary net productivity of forest vegetation is subject to internal factors such age and the type of tree cover (Santa Regina et al., 1997) and external environmental factors such as soil and climate. Annually, forestry plant retain only a part of their production in perennial structures, while its largest fraction falls to the ground as dead organic matter. Thus, litterfall provides the main input of C (primary input) as well as of nutrients to the forest floor. The amount of litterfall is related to climate and an inverse linear relationship between total litter production and latitude has been observed (Berg and Laskowski, 2006; Berg and McClaugherty, 2014). Primary input is added to the secondary input of C, represented by products of the animals and microorganism metabolism as well as of their bodies when they die.

Decomposition of both inputs contribute to losses. The loss of C from the soil is determined by the flow of CO_2 produced by autotrophic respiration (root respiration) and heterotrophic respiration of microorganisms that degrade the soil organic matter. However, CO_2 can be released also from sources such as abiotic chemical processes of oxidation and dissolution of carbonates (Burton and Beauchamp, 1994). In any case, the soil respiration exceeds all the other exchanges of C between soil and atmosphere. As the primary productivity, decomposition is also controlled by the climate as well as by biotic factors, such as soil organisms and litter quality. It is evident, therefore, that global change can profoundly affect both processes and especially the current balance between them.

Above and beyond what is actually the carbon stock in each forest and its distribution between the aboveground biomass and organic matter in soil, it is important to know the magnitude of C fluxes between the atmosphere and vegetation and between soil and atmosphere. This in order to better define sinks and sources.

Temperate and boreal forests, because of their past natural and anthropic disturbances, are relatively young with relatively high rates of growth (which imply carbon sequestration). The young age and exposure in certain areas with high levels of atmospheric CO_2 and nitrogen are such that they

behave as net carbon sinks (Dixon et al., 1994; Goodale et al., 2002; Nabuurs et al., 2003). Yet, it unknown for how long these ecosystems can keep behaving as sinks.

It seems that forests, once they reach a certain stability, they behave no more as a sink but they maintain a balance between emissions and revenue of C. In this regard, there are those who believe that European forests need another 50-100 years to reach this steady state (Kauppi et al., 1992) but others think that they will spend several more centuries before they reach the stationary state (Lugo and Brown, 1986).

In contrast to temperate and boreal forest soils, which are generally considered as mainly C sink, tropical forests can be seen as a source of CO_2. This source seems to be equivalent to almost 30% of annual emissions of CO_2 related to the use of fossil fuel (Brown and Lugo, 1990; Lugo and Brown, 1993). As previously discussed, the practice of forestry and afforestation and the application of good forest management practices can increase the stock of C in the forests, subtracting CO_2 from the atmosphere. However, in a view of global climate change, which was mentioned above, a question naturally arises: in which way the increase of atmospheric concentration of CO_2 and the consequent increase of world mean temperature will affect soil biological activity, the organic matter turnover and soil C stores?

Models on general circulation predict that the greatest temperature increase (4-6° C) will occur at high latitudes (Dixon et al., 1996; Giorgi and Lionello, 2008; Heywood, 2011). In boreal regions, a temperature rise would decrease the length of the winter reducing the permafrost areas (Davidson and Janssens, 2006).

An increase in atmospheric CO_2 can enhance net primary production. Nevertheless, in soils where inorganic N is limiting as well as in boreal and temperate regions, a CO_2 increase can modify the N distribution in plants and product a litter with a lower N concentration (Coûteaux et al., 1995) and therefore with higher C/N and C/lignin ratios. These ratios, as well as holocellulose/lignin and cellulose/lignin/N ratios, are indices that reflect the proportion of labile compounds (carbohydrates, proteins) and recalcitrant compounds (lignin, suberins, resins, fats and waxes) and would predict a slower rate of decomposition. In this view, few experiments have shown that a litter of poor quality, that is a low N concentration, slowly decomposed and would favour a higher C storage in the soil (Cotrufo et al., 2012; Hyvönen et al., 2007).

A temperature increase, on the other hand, could accelerate edaphic processes, especially biological ones, increasing litter degradation and nutrient cycling with a further increase of the release of greenhouse gases such as CO_2, CH_4 and N_2O that can cause a further increase in the global temperature. These effect could be more pronounced at middle and high latitude where the greatest temperature increase are expected (IPCC, 2013, 2001).

Nevertheless, the relationships between CO_2, temperature increase, plants and microorganisms responses are even more complex than it was supposed. Studies made in Finland along a mean temperature gradients and in the Great American Plains have suggested that the soil carbon pools

could decrease slower than expected according to models that only consider the effect of temperature increase on respiration (Liski et al., 2003).

In very dry or wet biomes, respectively with a very high or very low evapotranspiration to precipitations ratios (PET/P), the net soil carbon decreased when the mean annual temperature increased (Kirschbaum, 2006). Such a response, however, was not observed for biomes with PET/P ratios in the range 0.25–2. The absence or the limited carbon depletion evidenced in some ecosystems could depend on both the presence of physical-chemical C stabilizing reactions, accelerated by heating, that reduce microbial respiration and/or the soil litter decrease (Schulten and Leinweber, 2000; Thornley and Cannell, 2001).

The biodiversity of soil biota is also very important for the correct functioning of the ecosystem. In fact, the main of the metabolic processes occurring in soil can be carried out by various microbial species and so the disappearance of a given species usually have no significant influence on the overall processes. Nevertheless, some metabolic processes are carried out by a restricted number of microbial species and, in a case like this, their disappearance affect negatively this process. The presence or absence of such species, like nitrifying and ammonia oxidizing bacteria, as well as any variation of their abundance are index of the health state of the soil microbial community (Kowalchuk et al., 1998).

Most of the edaphic fauna, in mesic environments, is typically concentrated in the litter and in the upper soil layers, where the high organic matter content allows a high growth rate of the microbial community. The high adaptation of many groups of organisms of the edaphic fauna to soil, like those living in the partially decomposed litter layers, makes them unable to leave the soil when the environmental conditions become unfavourable to their survival. Such organisms, therefore, are particularly vulnerable to soil chemical-physical and biological changes, allowing them to be useful biological indicators of soil quality (Parisi et al., 2005), and are strongly affected by the climatic change.

Climate and soil quality changes also affect plant species diversity. The loss of biodiversity produces a considerable loss of both compositional and functional ecosystem characters compromising important processes such as efficient use of resources, productivity and resilience to disturbance (Tilman et al., 2001).

Hyvönen et al. (2007) give a thorough review of the impact of climate change, elevated CO_2 and higher N deposition on forest ecosystems. According to these authors, CO_2 increase causes higher photosynthetic rate, higher temperature lengthen the growing season and higher N deposition increase leaf area index. Accordingly, soil organic matter will simultaneously:

- Increase, with increasing litter input, although priming may decrease the soil C stock initially, but litter quality effects should be minimal (in response to N deposition and, partly, CO_2 response);
- Decrease, because of increasing temperature;

- Increase, because of retardation of decomposition with N deposition, although the rate of decomposition of high-quality litter can be increased and that of low-quality litter decreased.

The complexity of the ecosystem response evidences the necessity to cleverly study such a topic, mainly in the Mediterranean ecosystems, that are particularly vulnerable to climatic changes.

1.2 Plant litter decomposition: effects of climate, litter quality and microbial communities

Within forest ecology, a key role is played by the complex phenomenon of litter decomposition (Berg and Laskowski, 2006; Berg and McClaugherty, 2014; Swift et al., 1979) as only primary production can be seen as a more important ecosystem process (Moorhead and Sinsabaugh, 2006). Decomposition of plant litter is essential in the global carbon cycle, especially in boreal and temperate forests which, among other terrestrial ecosystems, are estimated to contain the largest pool of C in their soils (Dixon et al., 1994). Additionally, its role is also basic in nutrients' cycles and plant nutrition (Coûteaux et al., 1995; Guckland et al., 2009; Kaspari et al., 2008). Factors influencing litter decomposition are substrate quality, climate and soil biotic communities (Berg and McClaugherty, 2014).

There is a general consensus that, under unfavourable climatic conditions, climate has a higher importance in litter decomposition, while under favourable climatic conditions such as the Mediterranean climate, litter quality largely overcomes (Cortez et al., 1996). Moreover, at regional scale, climate (i.e. temperature and rainfall regimes) are the main drivers of litter decomposition (Liski et al., 2003) but at smaller scales litter quality may be a far more important driver (Berg and McClaugherty, 2014).

Substrate quality is particularly significant both in terms of nutrients translocation from plant to soil and vice versa (Sariyildiz and Anderson, 2005) affecting the rates of decomposition, mainly on the basis of nitrogen and lignin content (Fioretto et al., 2005a; Melillo et al., 1982; Taylor et al., 1989). Recently, also Mn has been shown as a very significant nutrient in decomposition ecology (Berg et al., 2006).

In further detail, quality depends on chemical and biochemical features of the litter, in terms of relative abundance of water soluble substances, polymer carbohydrates including pectin, hemicellulose and holocellulose, lignin and other aromatic compounds, lipids and waxes. C/N ratio, lignin/N, holocellulose/lignin, cellulose/lignin/N are indices that reflect the fraction of labile

compounds (carbohydrates, proteins) and recalcitrant compounds (mainly lignin, but also suberins, resins, fats and waxes) (Berg and McClaugherty, 2014; Berg et al., 1995; Rovira et al., 2008; Taylor et al., 1989). For a simplified, yet comprehensive diagram of the role of litter quality during decomposition, see Fig. 1-4.

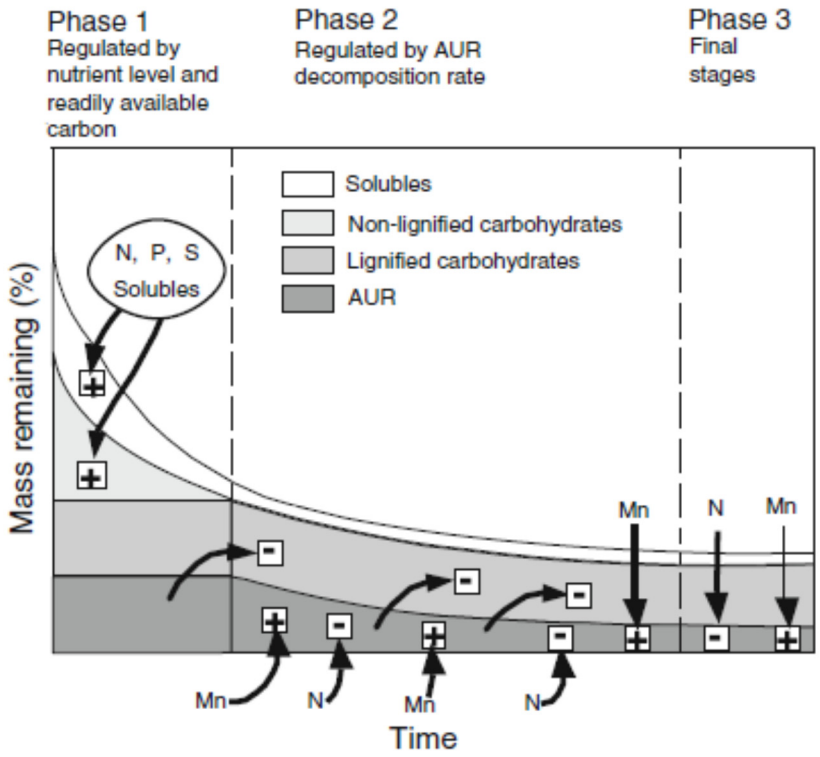


Figure 1-4: Generalized roles of different parameters of litter quality in pine needle litter. The decomposition of water soluble substances and unshielded cellulose/hemicellulose is stimulated by high levels of the major nutrients (early stage—phase 1). When degradable solubles and unshielded holocellulose are decomposed, only lignin-encrusted holocellulose and lignin remain as well as newly formed stable products. In the late stage (phase 2), the degradation of lignin (indicated as acid unhydrolyzable residue AUR) rules the litter decomposition rate. Nitrogen hampers the degradation of lignin, and higher N concentrations suppress the decomposition whereas higher Mn concentrations appear to have a stimulating effect on the degradation of lignin. Finally, in the humus-near stage (phase 3), the accumulated mass loss reaches its limit value at which the litter decomposition rate is close to zero. Adapted from Berg and McClaugherty (2014) and Berg and Matzner (1997).

Soluble substances and labile compounds are rapidly degraded in the early stage of decomposition by fast growing microorganisms that may require a high concentration of nitrogen (Swift et al., 1979). Cellulose and lignin, the most abundant components of litter, constituting 70-80% of fallen litter, are slowly decomposed. Nevertheless, because lignin physically protects most of cellulose and hemicellulose from enzymatic hydrolysis, neither group of compounds decompose independently (Cooke and Whipps, 1993). Each fraction has characteristic exponential kinetics of decomposition, so the total mass loss of litter is the sum of a number of exponential functions (Minderman, 1968). Decomposition process consists of mainly 2 phases: I) in the former phase, that lasts 1-2 years, the decomposition rate is regulated by the C and N availability, as well as litter morphological

characteristics (Melillo et al., 1982); II) in the latter phase, lasting more than 3 years, the rate is slow and regulated by lignin and Mn concentration, being such element essential for some lignin-degrading enzymes, such as Mn peroxidases (Berg et al., 2006). On the other hand, high lignin content has a rate reducing influence (Berg et al., 1996; Fioretto et al., 2005a) in particular, when associated with high N content, because new and stable complexes are formed (Berg and McClaugherty, 2014; Coûteaux et al., 1995).

Climate is also very important in the decomposition process, as it regulates the rates of decomposition in function of temperature and moisture regimes (Trofymow et al., 2002). It affects decomposition rate both directly, influencing the microbial activity and/or microbial species composition (Russell et al., 2007) and indirectly, altering the quality and the quantity of detrital inputs to soil and the chemical and physical processes that regulate soil organic carbon quality (Fissore et al., 2007).

Berg and Matzner (1997) reported for pine needle litter and some deciduous litter types along a climatic transect different effects of lignin with climate, possibly related to a difference in increase rate for N concentration in litter. Moreover, data suggest that under a less limiting climate, the fast growing fungi would have an advantage over more slowly growing lignin degraders. In colder climate, on the contrary, the lignin degraders would grow relatively better as compared to fungi degrading holocellulose (Berg and Laskowski, 2006). As a consequence, the accumulation of recalcitrant substances appear higher in a warm than in a cold climate although the overall litter decomposition rate may be higher in warm climatic condition. As the microorganisms are the actual degraders of organic matter, it is important to investigate how climatic and/or litter quality changes could affect microorganisms and their activity. This has been one of the most important and challenging problems in soil ecology (Panikov, 1999) and is even more important in consideration of global changes. It appears evident that an analysis of all the many facets of the decomposition process is important for an overall assessment of the effects of global change (Andersson et al., 2004) and many recent studies concerning decomposition and C cycle have taken into account which could be the consequences of the on-going climate change (Briones et al., 2010; Davidson and Janssens, 2006; Meier and Leuschner, 2010; Sariyildiz and Anderson, 2003).

As for soil biota, fungi and bacteria form unique communities and successions during the decomposition process (Berg et al., 1998; Romaní et al., 2006), with fungi as the main protagonists in the production of exoenzymes capable of lignin degradation (Osono, 2007). An example of fungi growing on decomposing beech leaf litter from the litterbags experiment done in this thesis (see in the following chapter) can be seen in Fig. 1.5.

Figure 1-5: Beech leaf litter decomposing into litterbags after 22 months in the field. In the upper part of the pictures, fungal hyphae can be seen.

Saprophytic microfungi, because of their hyphal growth pattern, production of vegetative spores, specific survival strategies and capacity to produce a great variety of enzymes important in the decomposition process, are ubiquitous and respond rapidly to the addition of new substrate (Kjøller and Struwe, 2002). They contribute up to 90% of the total respiration of soil organisms (Kjøller and Struwe, 2002) and a lot of them (Basidiomycetes) can attack the lignocellulose matrix which other organisms are unable to metabolize (Fig. 1-6). Because their different versatility, the composition of the fungal community changes during decomposition establishing a microbial succession (Schneider et al., 2012). Bacteria also contribute to litter decomposition by releasing a large variety of enzymes in the environment (Romaní et al., 2006). The study of the interaction between fungi and bacteria is important to understand the decomposition process. Nevertheless, in general, the fungal biomass exceeds the bacterial component such that fungi play the major role especially in the initial stage of cellulose, lignin and chitin decomposition (Swift et al., 1979).

In recent years, attention has turned to degrading capacity of microorganisms by evaluating their enzyme activities (Nannipieri, 2006). In fact, the decomposition rates of organic matter could depend from the enzymes degrading the main structural component of plant material (Fioretto et al., 2009; Sinsabaugh et al., 2005). In this view, the measure of degrading capacity of microorganisms could provide functional information on specific aspects and succession of the microbial communities during decomposition (Burns et al., 2013). Even though microbial communities release hundreds of different enzymes into the environment, those directly involved in the degradation of lignin and cellulose and in C, N, P and S cycles are of primary interest.

Degradation of the major structural constituents of plant organic matter requires multi-component enzyme systems, involving different microbial taxa. Cellulose, lignin and hemicellulose are covalently linked and physically intercalated in plant cell walls (Berg and McClaugherty, 2014) (Fig. 1-6). Thus, extensive degradation required concerted action by a variety of enzymes. Laccases,

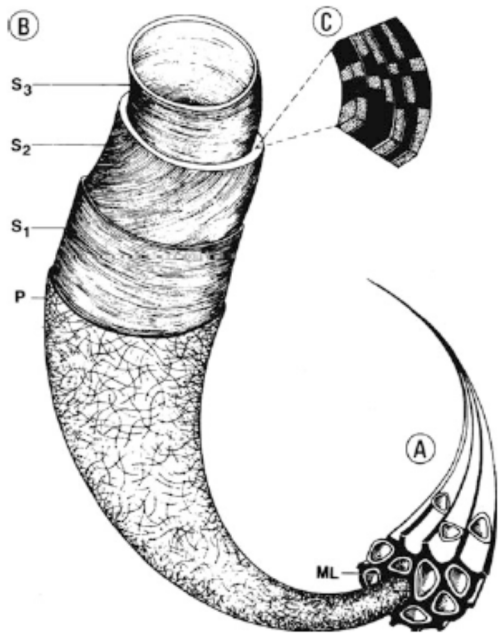


Figure 1-6: Overview of a plant fiber. A Tracheids. B Cell wall layers. C Arrangements of polymer carbohydrates and lignin in the secondary wall. Middle lamella (ML), primary wall (P), layers of the secondary wall (S1, S2, and S3). The model demonstrates the distribution of the lignin–hemicellulose matrix (black) hemicellulose (white) and cellulose fibrils (dotted). Adapted from Eriksson et al. (1990) and Berg and McClaugherty (2014).

released by numerous fungi, appear to be the best lignin degrading agents and act in synergy with other polyphenoloxidases such as Fe-peroxidases or Mn-peroxidases (Di Nardo et al., 2004; Tagger et al., 1998; Theuerl and Buscot, 2010). Cellulases, enzymes degrading cellulose, are divided at least in 2 enzyme classes: cellobiohydrolases (exocellulase) and endoglucanases (endocellulase), which exhibit different affinities to crystalline cellulose (Deng and Tabatabai, 1994). Even if the relationship between the enzyme activity and cellulose degradation is complex, an index of cellulase interactions, that is the product of exocellulase and endocellulase activities, seams correlated with the rate of disappearance of cellulose from decomposing leaf litter, both within and between species. Chitinases catalyse hydrolysis of chitin that is the main structural component of cell walls of most fungi and arthropods (Andersson et al., 2004). They are produced by many species of bacteria, streptomycetes and other actinomycetes, fungi as well as plants and play an important physiological and ecological role in ecosystems as recyclers of chitin, by generating C and N sources (Andersson et al., 2004; Ekenler and Tabatabai, 2002; Leatham, 1985).

Soil fauna, according to the size of the animals implicated, is involved in different parts of the decomposition process and soil quality (Knoepp et al., 2000). Soil macrofauna plays an important role in the initial comminution and degradation of plant litter. Detritivorous macro-invertebrates have both direct and indirect impacts on litter degradation (e.g. mechanical breakdown of dead leaves, biochemical modifications of organic matter during gut transit, mixing of organic and mineral particles, enhancement of microbial activity by increasing the available surface for microbial growth, physical disruption and/or dissemination of mycelial hyphae, etc.) (Hedde et al., 2007).

Even though most direct mineralization processes are carried out by soil microorganisms, soil invertebrates form an important part of the decomposer system and are responsible for changing nutrient mineralization rates and the spatial redistribution of nutrients with great relevance of groups as Collembola (Chamberlain et al., 2006) and Oligochaeta (Andriuzzi et al., 2013; Cortez and Bouché, 2001, 1998; Vliet et al., 2004).

1.3 The European beech (*Fagus sylvatica* L.) and its importance in soil C and decomposition processes

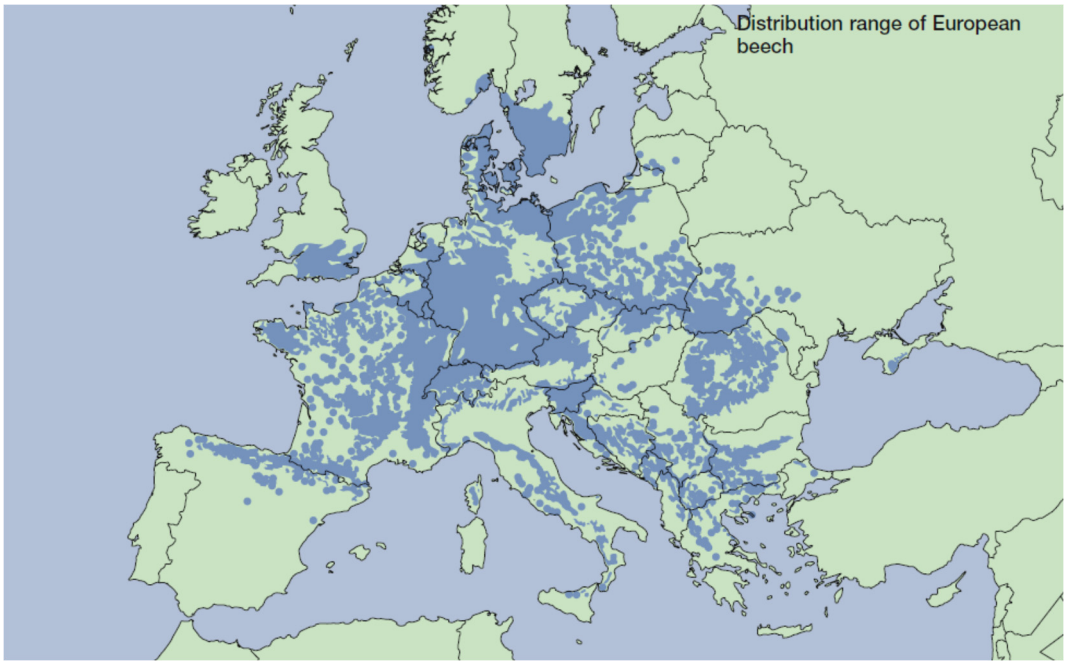


*Figure 1-7: Distribution of the European beech (*Fagus sylvatica *L.). Adapted from von Wühlisch (2008).*

One of the best studied plant in Europe is the European beech (*Fagus sylvatica* L.), which could be likely the most abundant plant in central Europe without human interference, forming mostly monospecific stands in a wide range of site conditions extending from humid to semiarid climates and from basic to acid soils (Ellenberg and Leuschner, 2010). The plant range extends from southern Sweden to northern Sicily, west to northern Portugal and central Spain, and east to northwest Turkey, where it intergrades with the oriental beech (*Fagus orientalis* Lipski), which is the allopatric species further east. For reasons of simplicity, from now onwards the European beech will be referred simply as *beech*.

The beech belongs to the family Fagaceae (along with other important genera like *Quercus* and *Castanea*). It is a tree not particularly long-living (200-300 years) that grows maximum 30-35 m tall, with a cylindrical trunk easily exceeding one meter in diameter.

Within the European context, the beech in peninsular Italy grows normally between altitudes of 800-1800 m along the Apennines (Pignatti, 1982), covering 9.4% of the country's total forest area (Nocentini, 2009) and accounting for up to 6.0 t C ha^{-1} yr^{-1} of carbon sequestration rate, similarly to tropical forests (Valentini et al., 2000). The species is present in all Italian regions except Sardinia. In southern Italy, beech stands are genetically distinct from those growing in the rest of Italy and Europe (Leonardi and Menozzi, 1996) and, because the Mediterranean climate is strongly affecting temperature and moisture regimes even at higher altitudes, during summer plants are adapted to longer periods of water stress (Amoriello and Costantini, 2000).

These forests have some floristic characters that make them well-differentiated compared to the beech woods of the rest of Europe. It is also possible to identify a good local diversity between the different districts of the Apennines (northern, central and southern the latter including also the northernmost mountain ranges in Sicily).

This broad distribution is the result of complex biogeographical events that have seen the beech alternately moving north-south along the peninsula during the Quaternary, in response to the alternating climatic conditions caused by glaciation (Feoli and Lagonegro, 1982). During the post-glacial periods, especially in the early stages when the climate was cooler and wetter, the beech took the upper hand over other forest stands, especially in mountain areas, as is also evident from the pollen diagrams (Paffetti et al., 2007). Central Italy, in particular, witnessed massive migration, as the territories of the Northern Apennines were recolonized from beech coming from the neighbouring districts of the central Apennines. The latter, in turn, must have come in contact several times with the southern populations, which were spreading northwards as much as southwards from their refuge locations on the mountains.

In this regard, looking at the most significant pollen diagrams, it is particularly interesting to notice the continuous presence of the beech in Italy since the Middle Pleistocene, with particularly significant concentrations around 200,000-170,000 years ago. From this point of view, the territories of central and southern Italy represented a unique area in Europe, as in other European regions *Fagus* seemed to be absent for long periods during the coldest moments. The beech was greatly expanding between 110,000 and 75,000 years ago, and it seems to persist in small, isolated populations between 75,000 and 15,000 years ago, at the peak of the last ice age.

The beech grows on both acid and mildly alkaline substrates, without any specific preference for a certain parent material. Accordingly, the Apennines show a strong lithological variability with a strong presence of sandstone, clay and marl in the Emilia-Tuscany Apennines, while the central and southern sections show a significant presence of limestone, but also, locally, of volcanic rocks (Lazio) or metamorphic rocks (Calabria), until reaching the lavas of Mount Etna. This lithological variability is reflected in differences in chemistry with limestone and dolomite, for example, originating basic substrates, while sandstones, which most of the times are rich in silica, originate acid soils.

Given its great importance in Europe as a forest species, there are several studies about the decomposition of beech plant material, in pure and/or mixed forests of Central Europe, and they have been carried out both in the field (Albers et al., 2004; Berg and Meentemeyer, 2002; D'Annunzio et al., 2008; Kooijman and Martinez-Hernandez, 2009; Melillo et al., 1982; Sariyildiz and Anderson, 2005; Vesterdal, 1999) and/or under laboratory conditions (Brandstätter et al., 2013; Cortez et al., 1996), yet there are very few studies investigating decomposition regimes in beech forests in the Apennines (Rutigliano et al., 1998, 1996, 1989). Many other studies are more related to the carbon stock in the soil and cycles of organic matter into these ecosystems (Guckland et al., 2009; Hedde et

al., 2008; Meier and Leuschner, 2010; Müller et al., 2009). Besides, being the Mediterranean region one of the most highly threatened by climate changes (IPCC, 2013), the impact of these changes on decomposition rates and C dynamics has not been investigated as much as in central Europe (Incerti et al., 2011).

1.4 Aim of the research

This Ph.D. thesis in **Applied Biology** focused on biology and ecology of leaf litter decomposition and quality and quantity of organic matter in several stands of beech forests along the Italian Apennines. The research presented here gave insights and new findings on organic matter cycles (including implications related to climate change) within beech forests along the Italian Apennines, which are important ecosystems that have not been comprehensively investigated on the point of view of decomposition ecology.

The research concentrated on both leaf litter (excluding wood materials lying on the forest floor) and soil. It has to be highlighted that the *soil* in this thesis has been studied strictly on a biological/ecological point of view and not always following the criteria and/or methods of pedology.

The results and discussions of this work are articulated in **three sections**, with the first two linked by the same study area (two beech stands north and south along the Apennines) and the third section considering the whole of the Apennines.

The **first section** of this research investigated the impact of litter/soil quality and climate on decomposition of beech leaf litter from two Italian locations (namely Pradaccio, in northern Italy and Laceno, in southern Italy). The section closely followed decomposition dynamics with the litterbags technique on-the-field. Moreover, nutrients dynamics (N, S, P, K, Ca, Mg, Fe and Mn) has been followed during decomposition, along with enzyme dynamics (namely cellulase and xylanase, laccase and peroxidase, acid and alkaline phosphomonoesterases, chitinase and dehydrogenase). Besides, a laboratory microcosm experiment has been set up to study the impact of climate on the decomposition for the same locations under controlled conditions.

The **second section** considered again the locations Laceno and Pradaccio. However, this section focused on the organic matter quality and quantity and enzyme dynamics within a decomposition continuum of the forest floor and the soil (until a depth of 40 cm). The analyses are followed during the seasons, from autumn to spring and summer (excluding the winter months due to snow cover).

The **third section** put attention to a north-south transect of 15 locations along the Italian Apennines. Within these locations, an estimate of quality and quantity of organic matter in both the forest floor and the soil has been given. Furthermore, a preliminary model for the amount of the organic matter for the whole beech distribution in the Italian Apennines has been created and discussed.

2 Materials and methods

Beech forest during winter on the Matese mountains (Campania, Italy).

2.1 Site description: Laceno and Pradaccio

Two locations have undergone in-depth seasonal analysis through both litterbag experiments (from December 2011 till October 2013) and seasonal samplings of their litters and soils (during October, May and July 2011). These two locations have been chosen according to their strong diversity in terms of soil characteristics and climate. One is located in the north of the Italian peninsula, while the other is positioned in the south (Fig. 2-1).

Figure 2-1: Two very contrasting general panoramas of Pradaccio and Laceno. Pradaccio (above) is shown during summer, with the artificial homonymous lake visible in the picture. Laceno (below) is shown in full winter in an exceptional cold and snowy period, with the homonymous tectonic-karst lake visible. Despite the appearances in these pictures, Pradaccio is on average much colder than Laceno.

The northern site, Pradaccio, is located on the northern slope of the Apennines, on the borders between the regions Emilia-Romagna and Tuscany, in the upper part of the Parma river valley, within a National Reserve called "Guadine-Pradaccio" extending for 289 ha surrounded by the National Park of the Tosco-Emiliano Apennine. The study plot is a beech stand at an elevation of approximately 1350 m a.m.s.l., with trees ageing between 70 and 80 years, growing on sandstone giving the soil a strongly acidic pH of 4.0 and a water holding capacity of 90 ml H_2O/100 g soil dry weight. The climate is oceanic, with mean temperature of 6.0 °C, averaging on 11.0 °C between April and September and 0.7 °C in the rest of the year. Snow cover is often very strong and lasting within late November to the beginning of May. Rainfall has peaks during autumn and spring, even though between June and August there is a sensible reduction of the rainfall. The total average rainfall amount is 2900 mm per year. Annual litter input is 2497 kg/ha.

The southern site, Laceno, lies close to a homonymous karst polje within the Regional Park of Monti Picentini, which extends for 62,200 ha in region Campania. The parent material is carbonate, giving the soil a mildly acidic pH of 5.5 with a water holding capacity of 120 ml H_2O/100 g soil dry

weight. The trees are on average the same age as those in Pradaccio, growing at approximately 1150 m a.m.s.l., with an oceanic climate strongly influenced by the Mediterranean climate of the surrounding areas. Between April and September, the average temperature is 13.7 °C and 3.7 °C in the rest of the year with an overall mean temperature of 8.7 °C. In accordance to the Mediterranean climates, there is a longer semiarid period (May to September) and precipitation peaks in spring and autumn, with an overall average rainfall of 2300 mm per year. Snow cover does not persist long and it is commonly present only between December and February. Annual litter input is 4313 kg/ha.

Pedological surveys done in the past years in the nearby areas acknowledged the soil from Pradaccio as Lithic Haploborolls according to Soil Taxonomy USDA with a loamy-sand to sandy-clay-loam texture (Leoni, 2008) while Laceno has been classified as Humic Haplustands according to Soil Taxonomy USDA with a loamy-sand to sandy-loam texture (Alvarez Romero, 2012).

A climate comparison for both a five years average and detail for the timespan of the litterbags field experiment duration can be seen in Fig. 2-2.

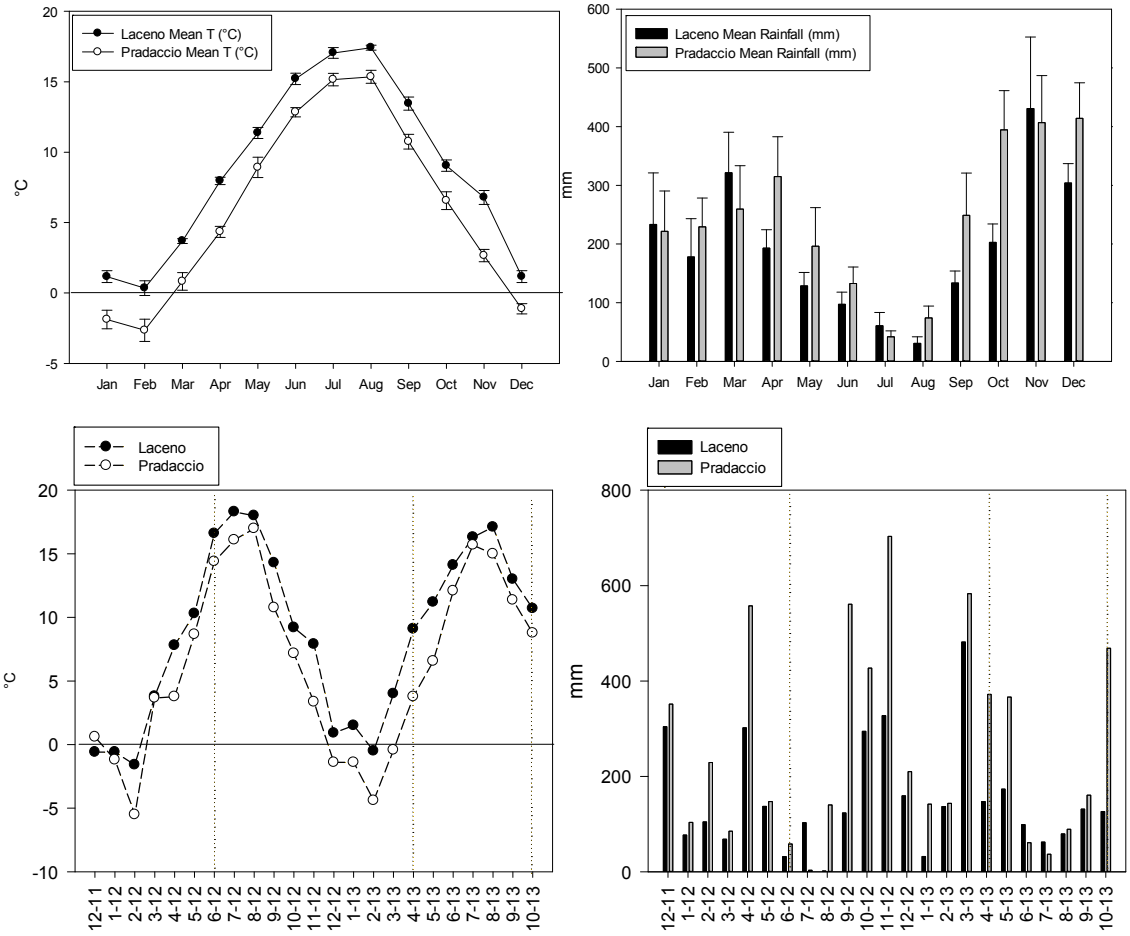


Figure 2-2: Climatic comparison between Laceno and Pradaccio. Above, a five years average can be seen (from 2008 to 2013). Below, detail data are shown for the duration of the field experiment (December 2011-October 2013) and sampling months are shown as dotted lines.

2.2 Site description: All other locations in the north-south Apennines transect

A total of 15 locations (including Laceno and Pradaccio) has been sampled within this project (Fig 2-3,4,5 and Tab. 2-1). The locations are distributed mostly in the central-southern part of the Italian Apennines and give a comprehensive image of the main beech districts within this mountain range. The northernmost location is Pradaccio (44° N), in Emilia-Romagna, while the southernmost is Nebrodi (Sicily). All of the 13 political regions that host beech stands have been sampled in at least one location, with the exception of Liguria, Umbria and Marche.

The stands have been chosen at different elevations in order to evaluate the effect of this factor on the organic matter quality and quantity. Thus, elevations between 1000 and 1600 m above mean sea level (amsl) are considered. Mean elevations of the sampling spots have been measured on the spot by means of a GPS (Garmin GPS Etrex 30). Two locations (namely Umbra and Vulture) stand

below the elevation of 800 m and in districts away from the Apennines; thus, they are considered heterotopic stands. Many locations have sedimentary parent material, mostly carbonate rocks like limestone or dolostone. Two locations only (namely Vulture and Serre Calabre) have igneous rocks as parent material. A set of topographical and ecological variables has been obtained by the WorldClim datasets (Hijmans et al., 2005). Some of these variables include topography features, like aspect or asperity index (as an index morphological heterogeneity). Other variables include temperatures and precipitations, with some derived feature, like isothermality (defined as the ratio between mean diurnal range and annual range of temperatures) or the amount of rainfall in the warmest quarter of the year.

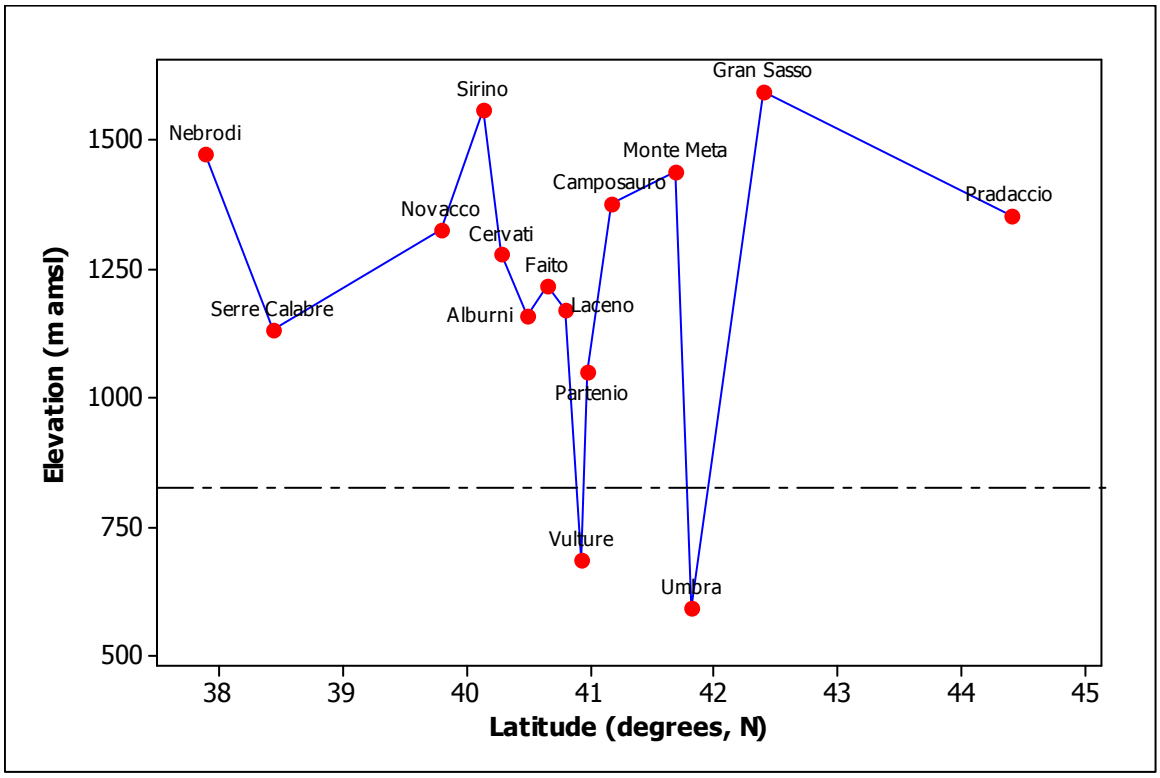


Figure 2-3: Distribution of the locations of the north-south Apennine transect according to their latitude and elevation. The dotted line represent the elevation of 800 m above mean sea level, which is conventionally considered the lower elevation limit for beech growth in Italian Apennine. All the locations lying below this elevation are considered heterotopic.

Table 2-1: Features of the 15 locations of the north-south Apennine transect studied in this thesis.

Location ID	Pradaccio	Gran Sasso	Umbra	Monte Meta	Camposauro	Partenio	Vulture	Laceno	Faito	Alburni	Cervati	Sirino	Novacco	Serre Calabre	Nebrodi
Region	Emilia Romagna	Abruzzo	Puglia	Molise	Campania	Campania	Basilicata	Campania	Campania	Campania	Campania	Basilicata	Calabria	Calabria	Sicilia
Parent Material	Sandstone	Limestone	Limestone	Limestone	Limestone	Limestone	Foidolite	Limestone	Limestone	Limestone	Limestone	Limestone	Dolostone	Granite	Marl
Latitude (degree N)	44,40	42,40	41,82	41,69	41,17	40,98	40,93	40,80	40,66	40,50	40,29	40,14	39,80	38,44	37,90
Longitude (degree E)	10,02	13,79	16,03	13,99	14,60	14,71	15,61	15,10	14,50	15,37	15,50	15,84	16,05	16,23	14,66
Elevation (m amsl)	1355	1596	593	1440	1376	1049	686	1170	1219	1161	1278	1558	1328	1134	1474
Asperity Index	405	690	161	288	538	650	578	449	599	178	540	514	493	233	283
Aspect (degrees)	29	92	35	97	154	41	279	27	256	213	48	10	282	333	130
T mean (°C)	6,4	7,3	12,4	8,1	8,8	10,4	12,5	8,7	9,9	9,7	9,4	7,4	9,1	11,3	9,7
Isothermality	23	27	27	27	26	27	29	25	26	26	26	24	25	29	25
Max T of Warmest Quarter (°C)	18,6	20,9	24,9	21,8	22,2	23,9	26,8	21,9	22,9	22,7	22,2	20,1	21,5	23,9	23,4
Annual Precipitation (mm)	2900	2700	1100	2400	1950	1800	1100	2300	2000	2000	2200	2600	2800	2900	1600
Soil average pH (H_2O)	4,00	6,48	5,82	5,84	5,44	6,19	6,32	5,70	6,20	5,73	6,20	5,50	6,15	5,45	5,68
Soil average Bulk Density (g/cm^3)	0,95	0,96	0,93	0,8	0,61	1,02	1,13	0,67	0,81	0,86	0,82	1,24	0,89	0,87	1,09

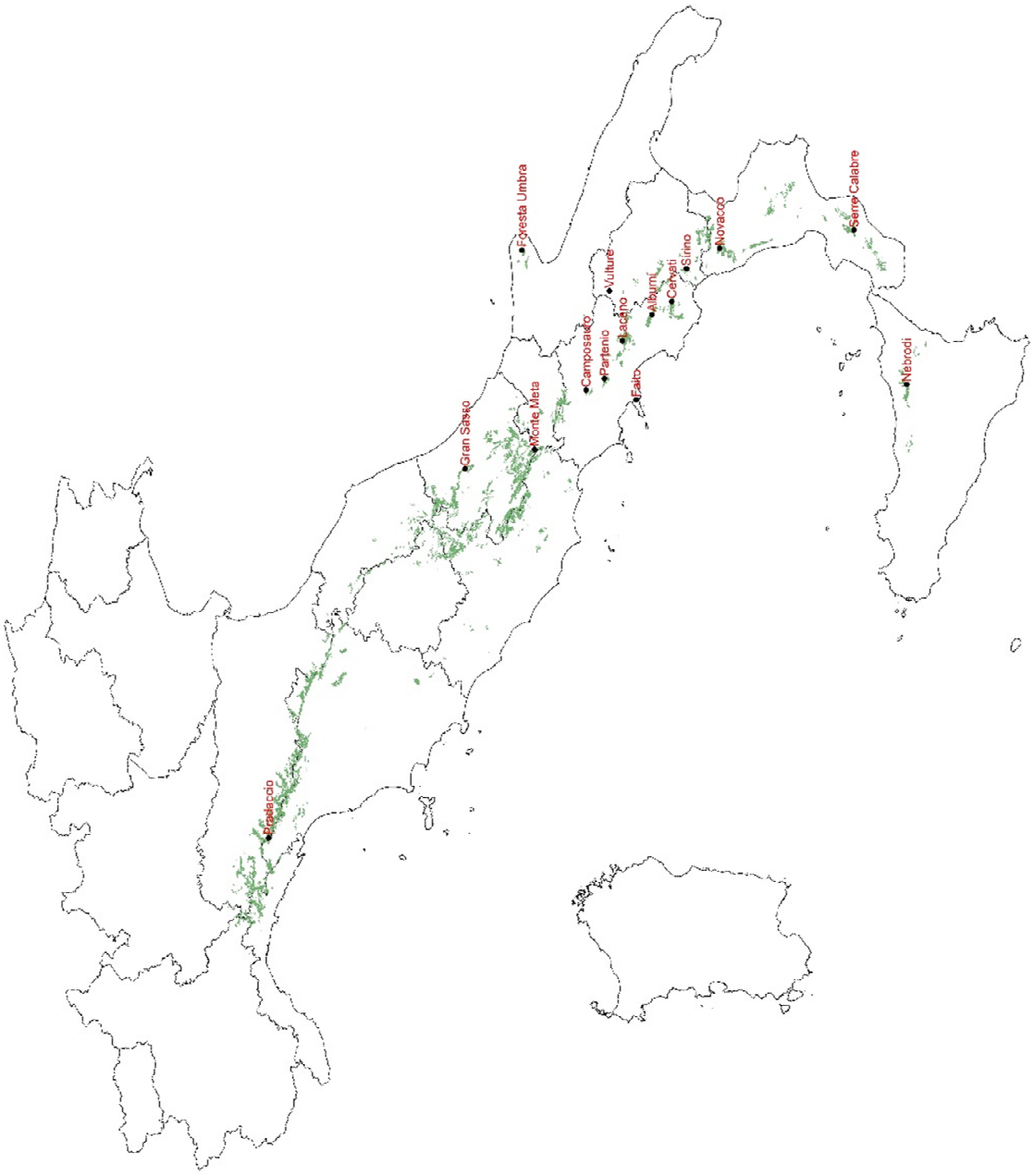


Figure 2-4: Map showing the distribution of the 15 locations studied in the north-south transect of the Italian Apennines. The green areas show the distribution of the beech along the Apennines (distribution on the Alps has been excluded). Scale is 1:5,000,000

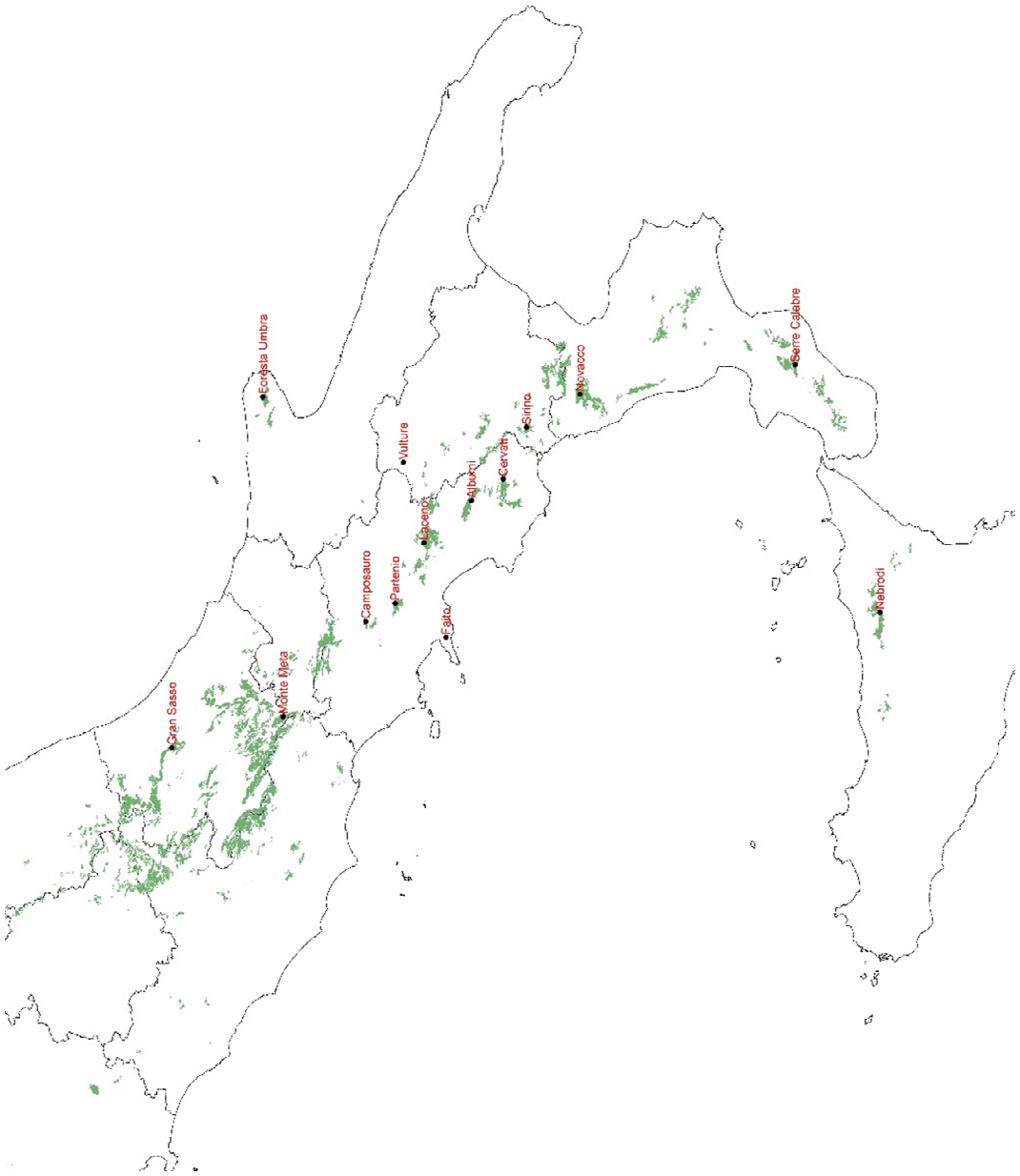


Figure 2-5: Detail of central-southern Italy showing the distribution of 14 locations studied in the north-south transect of the Italian Apennines. The green areas show the distribution of the beech along the Apennines (distribution on the Alps has been excluded). Scale is 1:3,000,000

2.3 Litter and soil collection, handling and processing

Per each location, to make a litter and/or soil **collection on the field**, 6 random replicates where chosen at a fixed distance between each other, in an area of maximum 1 ha. The area was selected with a homogenous tree density, without excessive presence of large stones, asperities, crevices etc. Being the beech forests in the Italian Apennines usually growing with non-homogenous topography, the 6 replicates where done 2 in flat areas, 2 in gentle slope areas (0-5°) and 2 in middle slope areas (5-10°) along a north-south transect within the chosen hectare, in order to evaluate more thoroughly the area in its complete topography. Areas with slope exceeding 10° were discarded because of an excessive effect of topography on soil and litter amounts.

Figure 2-6: Sampling of litter (left) and soil (right).

Litter has been sampled by placing a 20x20 cm square steel frame on the forest floor. All the forest floor was then transferred into plastic bag (excluding growing saplings, when present) harvesting everything until any plant-derived material was morphologically recognizable (Fig. 2-6 left). The material was then transferred into plastic bags and kept under ice until processing.

Soil was sampled with a steel core sampler with a diameter of 5 cm and a length of 40 cm, which can be opened into two halves. Cores made of polymethyl-methacrylate, sealed with paper tape, when then transferred into one half of core sampler and then closed within the other half. The core sampler was placed in the hole left by the litter harvesting and the soil core was obtained by hammering the core sampler in the soil, avoiding over-hammering in order to reduce potential compression of the soil within the core (Fig. 2-6 right). Subsequently, the core was taken out from the core sampler and sealed into plastic tubes and kept under ice until processing.

All the material was transferred in the laboratory. When sampling were done in locations which were too far away from the laboratory (located in the **Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Caserta**, Italy), the material were kept under ice or at 4 °C. Anyway, the samples were always transferred to the laboratory within 48h from the sampling itself.

Once in the laboratory, litter and soil were immediately processed. Litter was weighted before and after the removal of all non-leaf litter material. Then, the leaf litter was divided into layers according to the degree of fragmentation/fermentation to create a **decomposition continuum**. The criteria adopted for the layers are:

- **Li**: leaf litter where the leaves do not show any evident sign of fragmentation and/or chemical alteration. It might include mostly more or less newly shed leaves, but also older leaves which have not yet been visibly altered. Leaves in this layer are easily separated from each other;

- **Lf**: leaf litter with evident signs of fragmentation and/or chemical alteration but where the original structure of the leaf itself is still recognizable. Leaves in this layer can be separated from each other;

- **Lhf**: leaf litter with very strong signs of fragmentation and/or chemical alteration, where the original shape of the leaves is hardly recognizable. Leaves in this layer are usually impossible to separate from each other and are most commonly sealed together by fungi mycelia.

The three layers were then reweighted separately and a subsample was oven dried at 75 °C for 48 h in triplicate to assess the moisture content. Other aliquots were kept at -80 °C and others were air dried.

Soil cores where carefully opened and the core within was labelled and photographed before any other operation. Layers of the soil cores have been separated not according to pedological criteria, because for not all of the locations soil profiles were available and/or possible to evaluate within the times and possibilities of this project. Thus, in order to standardize and compare the results from the different locations, the cores were divided in layers of unequal lengths, namely 0-5 cm, 5-15 cm, 15-30 cm and 30-40 cm (Fig. 2-7). The 0 in the soil cores began exactly when no more leaf litter was recognizable. Thus, with some cautionary words concerning the great variability of Italian forest soils, it can be generally stated that the 0-5 cm layer of this thesis generally corresponds to parts of an O horizon or what in other studies is called H (humus), whereas the deeper layers, especially after 15 cm, could correspond to A or B horizons of mineral soils. Anyway, no pedagogical characterization is claimed within this study.

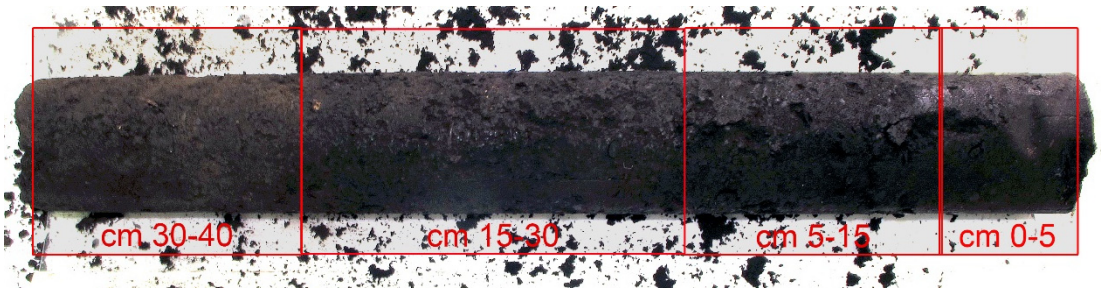


Figure 2-7: Example of an opened soil core with the four layers identified according to their depth.

After the cores were cut into the four layers, they were weighted, sieved with a mesh size of 2 mm and then weighted again to assess the percentage of gravel within the sample. A subsample was oven dried at 105 °C for 48 in triplicate to assess the moisture content. Other aliquots were kept at -80 °C and others were air dried.

The corresponding dry weight of the soil cores was used also to compute the **bulk density**, expressed as g of dry soil within the soil core, equivalent to cylinders of volume of $l\pi r^2$, with r (radius of the core sampler) being 2.5 cm an l (length of the core) variable between 5, 10 and 15 according to the length of the considered layer.

2.4 Litter traps, litterbags and microcosms

The litterbag experiment, divided into an on-the-field and a laboratory microcosm experiment, has been done within the locations Laceno and Pradaccio.

Between the second half of September and the end of November 2011, **newly shed leaves** have been collected on a surface of 1 ha in the two forests with 6 equally distanced nets suspended from the ground through plastic circles of 80 cm Ø. Samples have been collected several times until complete litter fall, taken in the lab where they have been air dried for two weeks and then subsamples have been oven dried at 60 °C for 48 h, weighted and then stored at 20-25 °C inside plastic bags. This samples allowed also to compute the **annual litter input**. This study, in all its sections, will always consider only the **leaf litter excluding all wood/woody material**. The reasons for this choice are: a) the leaf litter input has been measured always as more than 80% of the total input; b) the decomposition of leaf and wood/woody material proceeds in very different times and with different microbial communities and c) the enzyme activities were not easily measurable on wood/woody material for logistic and technical reasons.

For the **field experiments**, terylene litterbags of 20×20 cm with a mesh size of 1.5 mm^2 have been prepared, which allows interaction with most of the mesofauna except the largest animals (Joergensen et al., 2009). Each litterbag has been filled with approximately 4 g of dried newly shed litter. 288 litterbags with either leaf material of Laceno or Pradaccio have been prepared and then half of them has been set for decomposition in Laceno forest and the other half in Pradaccio forest. In each forest, 6 randomized locations have been chosen and in each of them 24 litterbags from

Laceno and 24 litterbags from Pradaccio have been laid on the surface of the organic soil, nailed, enclosed for protection with a plastic net with a mesh size of 10 mm^2 and then covered with leaves and wood material coming from the forest floor. Litterbags have been incubated in December 2011 in both sites and collected three times at 200, 500 and 680 days of decomposition. Each time, 3 litterbags per each leaf material of the 6 locations in the two forests have been harvested. Samples could not be collected after one year of incubation because of the heavy snow falling in the site of Pradaccio in December 2012.

Figure 2-8: Litterbags at their first day of on-the-field decomposition before they were covered with material coming from the forest floor.

For the **laboratory microcosm experiments** and chemical/physical measurements, organic soil down to a depth of 5 cm has been collected in the two forests in 6 random positions after removing the upstanding forest floor material (leaves and twigs more or less decomposed) collected in a separate plastic bag. The material has been immediately sent to the **Institute of Plant Nutrition of the University of Hannover**, Germany, pooled together and kept at 15 °C and constant soil humidity of 60% for one week to preserve the soil communities alive and let them adapt to the experimental conditions. In order to maintain the soil as much as possible similar to the field condition, it has not been sieved, and only the largest stones or roots have been manually removed. It has to be pointed out that the soil from Pradaccio contained a substantial biomass of earthworms, likely belonging to the genus *Aporrectodea*, whereas this was not observed for Laceno.

Microcosms have been prepared by adding approximately a corresponding dry weight of 1.7 kg of soil into 24 plastic boxes (42×35×18 cm), 12 per either soil. The boxes have been covered with a lid to prevent excessive evapotranspiration but small holes have been made on the side of the boxes

to facilitate gas exchanges. In each box, 8 terylene litterbags of 17.5×10.5 cm with a mesh size of 1.5 mm^2 have been placed, containing about 2.5 g of newly shed litter, half of them with plant material from Laceno and the rest from Pradaccio. After setting all the boxes, the litterbags have been moistened by spraying about 200 ml of deionized water on each litterbag. Successively, the litterbags lying in the boxes have been covered with a layer of plant material coming from the forest floor of either forest according to the soil type contained in the box (Fig. 2-9). Moistening during the rest of the experiment has been done, for the litter, by spraying 50 ml of deionized water on the litter material every three days and, for the soil, by adding the required amount of deionized water to keep a constant weight corresponding to 60% of soil water content. Of the 24 boxes, 6 of them (3 per each soil type) have been kept under control condition (constant temperature of 15 °C for 90 days and constant soil moisture of 60%). Additional 6 boxes underwent temperature modifications, with 15 °C for the first 30 days, 22 °C from 30 to 60 days and 8 °C from 60 to 90 days, keeping the soil humidity constant at 60%. Another 6 boxes underwent water regime changes, keeping the soil moisture constant to 60% for the first 30 days, but stopping any water addition from day 30 to day 60 and resuming watering from day 60 to day 90, keeping the temperature constant at 15 °C. Samples have been collected after 30, 60 and 90 days of the experiments, harvesting 4 litterbags per each leaf material, box and condition at a time. Decomposition in microcosm condition has already been proved to be extensively faster than the one on the field (Cortez et al., 1996; Taylor et al., 1989).

Figure 2-9: Microcosms with soil, litterbags and forest floor material within the climate chambers of the University of Hannover.

2.5 Mass loss determination and decomposition rates

After collection, each litterbag has been opened and carefully cleaned with a soft brush in order to remove large fauna and particles of soil. Afterwards, the litter has been dried at 75 °C for 48 h and only then weighted. Considering M as the dry mass in g of each litter bag and t_x as the days of decomposition, **residual weight** has been measured as:

$$Residual\ Weight = \frac{M(t_x)}{M(t_0)} \times 100$$

The decomposition trends have been evaluated according to the single exponential model (Olson, 1963). The model suggests decomposition at a constant rate, given by the decomposition constant k value. It can be seen as a three-phase model, with a fast initial phase, nutrient-regulated initial decomposition linked to leaching and degradation of easily degradable solubles and C sources, while the later stages are more regulated by lignin and complex compounds between lignin and N until a stable phase is reached (Berg and McClaugherty, 2014; Berg et al., 2010, 1996; Coûteaux et al., 1995). Given M as the mass remaining at the x time (T), the **decomposition constant** k (days^{-1}) is obtained from:

$$k = -\left(\ln \frac{M(t_x)}{M(t_0)} \times \frac{1}{t_x}\right)$$

2.6 Chemical analyses – soil and litter

For the soil, **pH** has been measured by shaking 10 g of soil in 25 ml distilled water for 10 min (Fioretto et al., 2000). After waiting 10 min, the supernatant was used to measure the pH with an electronic pH meter (HI 8424, HANNA Instruments, Sarmeola di Rubano, Italy).

Organic matter was evaluated gravimetrically as the difference between the dry weight and the ashes after incineration in a muffle furnace. For the litter, finely ground samples were burnt at 550 °C for 4 h into oven dried porcelain capsules (Fioretto et al., 2005a), while for the soil the temperature used was 375 °C for 16 h (Pribyl, 2010). The differences in temperatures are due to the risk of partial calcination reactions and/or loss of structural water from the mineral, and thus organic matter overestimation, when heating the soil at temperatures too high.

To assess **nitrogen mineralization** (N_{min}) within the laboratory microcosms, a soil incubation experiment has been designed by collecting subsamples of soil from the two locations. Successively, samples have been air dried and sieved (2 mm) and 50 g have been put into small plastic beakers and kept at 15 °C and 50% soil moisture content for 90 days, harvesting three beakers for each site every 30 days. N_{min} has been followed by monitoring contents of NO_3^-–N and NH_4^+–N which were determined by shaking for 1 h 50 g of fresh soil into 200 ml of 0.1 M KCl (Behrens, 2011). After

filtration, analytical measurements have been done through a colorimetric reaction with an autoanalyzer (Technicon Autoanalyzer II, Brahn und Lübbe, Norderstedt, Germany).

On field soil, after air drying and sieving, **phosphorus** has been evaluated by the Olson $NaHCO_3$ method (Frank et al., 1998). **Exchangeable K, Ca and Mg** have been estimated through an extraction of 2 g soil intro 20 ml with 1M pH 7.0 NH_4OAc (Warncke and Brown, 1998) with subsequent filtration and analytical determinations by inductively coupled plasma atomic emission spectrometry (ICP-AES) without ultrasonic nebulization.

Carbon, nitrogen and sulphur were measured by a CNS elemental analyser (vario El III, Elementar Analysensysteme GmbH, Hanau, Germany) after the dried samples were finely ground (Song et al., 2012) and carbonates removed from the soil samples. For litter **P, K, Ca, Mg, Mn and Fe contents**, finely ground samples were dry-mineralized in a muffle furnace at 480 °C for 16 h. Ashes were rehydrated with 1 ml 1:3 HNO_3 and 9 ml double-distilled water. After waiting 90 min, samples were filtered and then analytical determinations were performed by ICP-AES without ultrasonic nebulization (Bussotti et al., 2005).

Cellulose and lignin were evaluated according to the method of Van Soest and Wine (1964) with modifications (Fioretto et al., 2005a). Briefly, aliquots of oven-dried fine powdered samples (350 mg), were transferred to Falcon tubes (50 ml) and suspended in a sulphuric acid (0.5 M) and cetyltrimethylammonium bromide (CTAB) (20 g/l) solution. The tubes were kept in a water bath at 100 °C for 1 h. After cooling, they were centrifuged. The pellet material, called acid– detergent–fibre (ADF), was collected, washed in 30 ml of hot distilled water and 20 ml of sodium hydrogen carbonate (0.01 M) and centrifuged until the supernatant pH was higher than 6 (3–4 times). After that, the pellet was washed with acetone, centrifuged, oven dried and weighted. The ADF contained lignin, cellulose and some ashes. The bicarbonate ($NaHCO_3$) is used to remove residual sulphuric acid and then return the pH to values close to neutrality. The acetone wash serves, instead, to faster dehydrate the sample. Subsequently, the samples were placed in an oven at 75 °C until their weight was stabilized and only then weighted.

Aliquots of ADF were transferred to Falcon tubes and oxidized by adding a buffered permanganate solution, obtained by mixing two parts of saturated potassium permanganate solution (50 g/l $KMnO_4$ and 0.05 g/l Ag_2SO_4) and one part of lignin buffer (6 g/l $Fe(NO_3)_3$•9H2O and 0.15 g/l $AgNO_3$, 0.5 l/l glacial acetic acid, 5 g/l potassium acetate, 0.4 l/l methylpropan-2-ol). The oxidation was performed at 20–25 °C for 90 min. After centrifugation, the pellet was treated with a demineralising solution (50 g oxalic acid dihydrate in 0.5 l concentrated HCl and 0.7 l ethanol 95%) until complete decolouration. The suspension was centrifuged and the pellet was washed twice by 95% ethanol. The acid–detergent–fibre-after-oxidation (ADFAO) pellet was washed with acetone, centrifuged, oven dried and weighted. Cellulose was determined by weight loss of the ADFAO upon ashing at 550 °C for 4 h. Lignin was determined by subtracting the ADFAO organic fraction from the ash-free ADF mass. Lignin and cellulose contents were expressed as percentage of organic matter

present in the original sample. It has to be stressed and highlighted that this method gives an estimate of the proximate holocellulose (celluloses + hemicelluloses) and of lignin and lignin-like substances. In particular, lignin and lignin-like substances are often known and/or equivalent to Klason lignin (Sjöberg et al., 2004), acid unhydrolyzable residue (Berg and McClaugherty, 2014) or acid insoluble residue (Hilli et al., 2008). Nevertheless, keeping in mind what is their actual meaning, for reasons simplicity from now on in the text it will be referred to them simply as cellulose and lignin.

2.7 Assays of potential enzyme activities

Cellulase EC 3.2.1.4 and xylanase EC 3.2.1.8 were determined according to Schinner and von Mersi (1990). The method exploits the formation of a soluble form of Prussian blue ($KFe\{Fe(CN)_6\}\bullet H_2O$) under acidic conditions using the reducing power of sugars (equivalent to glucose) for the redox reactions necessary for the colour development (Adhikamsetty and Jonnalagadda, 2009). For the litter material, an extraction of the enzymes in sodium acetate buffer 50 mM pH 5.5 was necessary, while for the soil a direct approach has been used. The method used carboxymethylcellulose and xylan as substrates of the two enzyme reactions (cellulase and xylanase, respectively), and the reactions products are considered to be glucose equivalent. The molar absorption coefficient used was 30,000 M^{-1} cm^{-1} at 690 nm. Results are expressed as µmol Glucose equivalent / g Organic Matter / h.

Laccase EC 1.10.3.2 and peroxidase EC 1.11.1.x were determined according to Leatham and Stahmann (1981) with modifications (Di Nardo et al., 2004). The method exploits the oxidizing power of the enzymes to oxide 2-amino-1-methylbenzene (*o*-toluidine, C_7H_9N) for laccase and 2-amino-1-methylbenzene + H_2O_2 for peroxidase into a blue coloured product within a reaction of 2 min. Longer reactions (under laboratory condition >12 h) would further oxide the products to melanin, which is a product from lignin degradation by fungi (Osono, 2007). For both litter and soil an extraction with sodium acetate buffer 50 mM pH 5.0 was necessary. The molar absorption coefficient used was 6,340 M^{-1} cm^{-1} at 600 nm. Results are expressed as µmol *o*-toluidine oxidised / g Organic Matter / h. It has to be noted that the method for peroxidase does not allow to distinguish between lignin-peroxidase EC 1.11.1.14 or Mn-peroxidase EC 1.11.1.14 or other forms of peroxidase that may be present in the litter or the soil. Nevertheless, the most common form associated with ligninolytic fungi is Mn-peroxidase, which catalyses the oxidation of Mn^{2+} to Mn^{3+} in the presence of H_2O_2 under acidic circumstances (Palma et al., 2000; Šnajdr et al., 2008a).

Acid EC 3.1.3.2 and alkaline EC 3.1.3.1 phosphomonoesterases were determined according to Eivazi and Tabatabai (1977). The method exploits the formation of 4-Nitrophenol (also known as para-Nitrophenol, pNP, $C_6H_5NO_3$) from 4-Nitrophenylphosphate ($C_6H_5NO_3P$). The two assays are similar, but a modified universal buffer pH 6.5 or pH 11 is used for either acid or alkaline phosphomonoesterase. An extraction was necessary for the litter, while for the soil a direct approach

has been used. The molar absorption coefficient used was 178,000 M^{-1} cm^{-1} at 405 nm. Results are expressed as µmol pNP / g Organic Matter / h.

Chitinase EC 3.2.1.14 (also known as poly[1,4-(N-acetyl-β-D-glucosaminide)] glycanohydrolase) was determined according to Verchot and Borelli (2005). The method exploits the formation of 4-Nitrophenol from 4-Nitrophenyl-N-acetyl-β-D-glucosaminide. For the litter, it was necessary an extraction of the enzymes in sodium acetate buffer 50 mM pH 5.5, while for the soil a direct approach has been used. The molar absorption coefficient used was 178,000 M^{-1} cm^{-1} at 405 nm. Results are expressed as µmol pNP / g Organic Matter / h.

Dehydrogenase EC 1.1.1.x was determined according to Von Mersi and Schinner (1991). The method exploits the formation of iodonitrotetrazolium violet-formazan (INTF) from iodonitrotetrazolium chloride (INT). For both litter and soil the use of a direct approach was possible. The molar absorption coefficient used was 18,400 M^{-1} cm^{-1} at 464 nm. Results are expressed as µmol INTF / g Organic Matter / h.

2.8 Statistics and Geographic Information System (GIS)

Parametric descriptors like mean, standard deviation and standard error have been used as descriptors of central tendency and variability. When necessary, the median and its 95% confidence interval were chosen as descriptor, but in all cases this will be pointed out in the text clearly.

All data have been inspected for outliers and those have been removed when necessary. Data have been checked for normality and heteroscedasticity and, when necessary, in order to perform parametric statistics, they have been log-transformed or power-transformed. The base for the logarithm transformation was always 10. The power-transformation used was the Box-Cox transformation with the parameter λ chosen case by case. At any rate, whenever data have been transformed it is clearly stated in the text.

For correlation, either Pearson or Spearman's rank correlation coefficient have been used. Spearman's rank correlation was more generally used, as many data were having non-linear relationships.

Differences between two groups have been done with a 2-sample T-test, while differences between more than two groups, with two or three factors explaining the variance, have been sampled with a Two or Three-Way ANOVA.

Regression analysis have been done through use of linear models, general linear model or polynomial regressions. When necessary, regressions have been done on transformed variables. Analysis of residuals, although not shown in the text, has always been performed to assess the validity of the models.

Multivariate analysis included Principal Component Analysis, performed always with the correlation matrix and showing the results from the first two principal components only.

All graphs and statistical analyses have been done with SigmaPlot 12.0, Minitab 16.0 and the latest version of the software R.

For the GIS analyses, the software QGIS was used, while the cartography used was the CORINE Land Cover dataset and the WorldClim Dataset.

3 Results from the litterbags experiments of Laceno and Pradaccio

Beech forest of Laceno in winter.

3.1 Mass loss and decomposition constant

On the field, the decomposition trends of all the litter bags are comparable, with an average residual weight of proximately 65% at the latest sampling time (almost two years of field decomposition) (Fig. 3-1). The decomposition constants *k* are clearly different according to the two sites and they are higher for Laceno than for Pradaccio (Tab. 3-1). The trend is more stable and uniform for the location Laceno, whereas in Pradaccio the plant material decomposing behaves in a different way. However, it can be noted that, in either site, the plant material of Pradaccio decomposes faster than the one from Laceno.

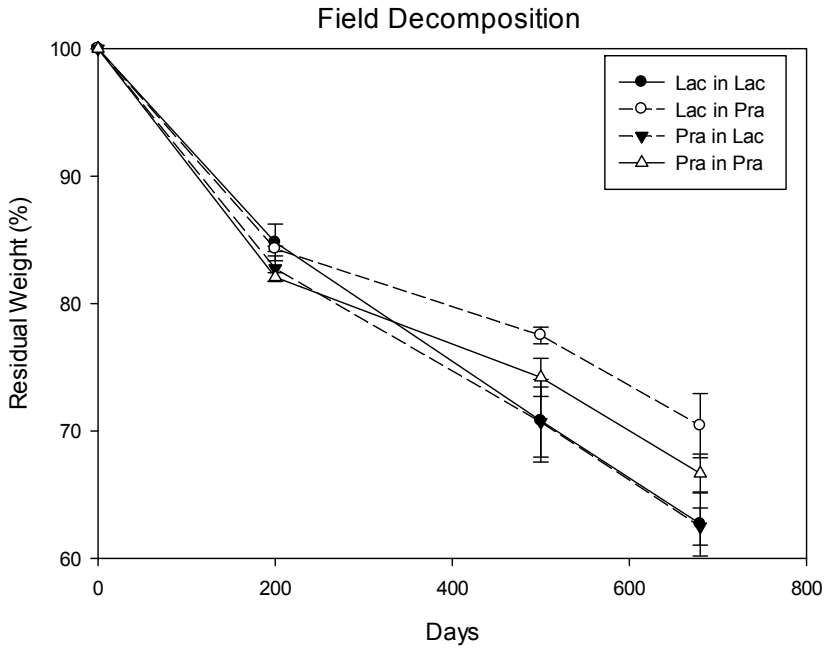


Figure 3-1: Field Decomposition. Values are means±s.e. (n=6)

Table 3-1: Decomposition constants and time estimated to reach 50% of initial mass.

Plant Material	Location	k (days^{-1})	T 50% Residual Weight (days)
Laceno	Laceno	0.00069	1009
Laceno	Pradaccio	0.00051	1348
Pradaccio	Laceno	0.00069	1001
Pradaccio	Pradaccio	0.00059	1166

A Two-way ANOVA repeated for the three sampling moments reveals that, at the early stage of the decomposition, the factor expressing 93% of the variance is Plant Material, whereas from the middle steps of the decomposition process till the later stages, factor Location becomes significant and expresses around 80% of the total variance (Tab. 3-2). It is clear, thus, that although the different quality of Plant Material plays a role during decomposition, after the early stages climate, pH, microbial communities and other proprieties of the two locations exert a greater role. The interaction

between Plant Material and Location is never significant, although it can be noted that its F and *p* statistics tend to increase in time.

Table 3-2: Results of Two-Way ANOVA for the variable Residual Weight in the three sampling moments of the on-the-field decomposition. Values that are statistically significant are bold.

Sampling	Source of Variation	F	*p*
200 days	Plant Material	5.67	**0.027**
	Location	0.43	0.521
	Plant Material × Location	0.01	0.924
500 days	Plant Material	0.51	0.483
	Location	4.69	**0.043**
	Plant Material × Location	0.49	0.491
680 days	Plant Material	0.93	0.345
	Location	8.21	**0.010**
	Plant Material × Location	0.72	0.406

Under laboratory condition, the decomposition process proved to be noticeably faster (Fig. 3-2). The results from the on-the-field experiment are directly comparable only with the last sampling of the laboratory experiment, while the other samplings (30 and 90 days) allow us to follow the very early stages of decomposition, which were not sampled in the actual forests. As a matter of fact, under Control condition (i.e. constant temperature of 15 °C and constant soil moisture of 60%) the residual weight after 90 days was on average 82%, which is a number 3 to 4 folds larger than what could be expected by the on-the-field decomposition constant. It suffice to notice that a residual weight of approximately 80% is reached, on the field, after 200 days of decomposition, while the laboratory experiment lasted only 90 days. In the other conditions, at both 60 and 90 days (i.e. when something was changed in temperature and/or soil humidity), the mass loss was usually lower than the Control condition, implying a differential response of microbial communities (Fig. 3-3).

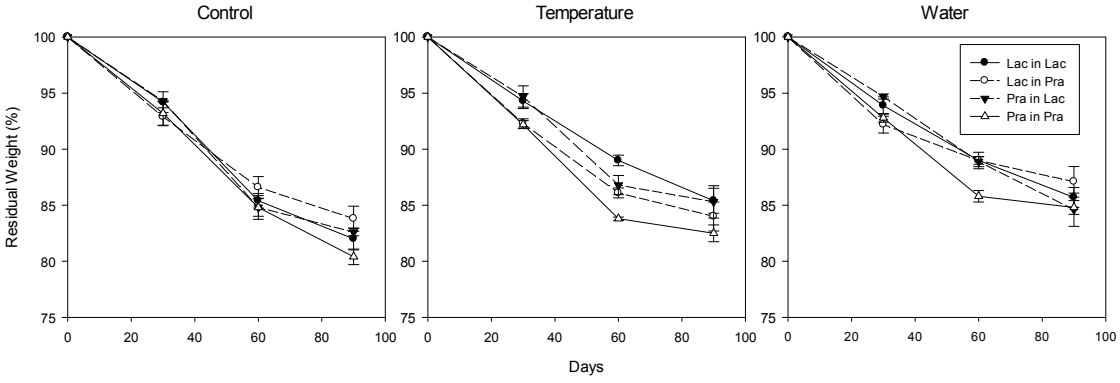


Figure 3-2: Laboratory decomposition in the three conditions. Values are means±s.e. (n=4)

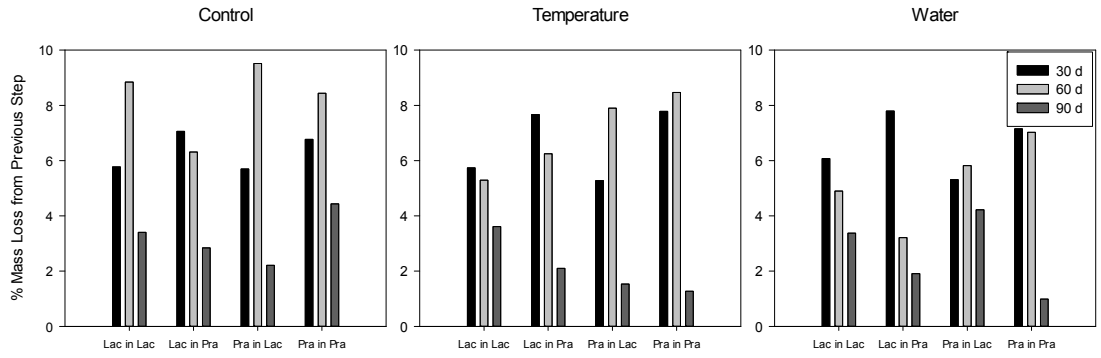


Figure 3-3: Percentage of mass loss between the different steps of the experiment in the three conditions.

To get into the details of the decomposition process in the microcosms, the results from a Three-Way ANOVA during the three steps of the experiments are shown in Tab. 3-3. During the first 30 days of decomposition, being both temperature and soil humidity constant, there is no real effect from the Condition factor. Similarly, the different quality of the Leaf Material does not have a substantial effect. Only the factor Soil is highly significant, explaining more than 90% of the variance. In fact, for the first 30 days, leaves decomposing on the soil of Pradaccio have 1.7% more loss of weight, and this trend appears to be in apparent contradiction to the field data.

After 60 days, the situation is more complex. The highest variance (32% of total) is explained by the factor Leaf Material, but also factor Condition explains a significant 29% of total variance. Factor Soil, by itself, now explains only 18% of the total variance, but there is also a significant interaction of Leaf Material and Soil, explaining 6% of the variance. This implies that, altering the main climatic features of temperature and humidity, a very multifaceted interaction between litter quality and the soil microbial communities is triggered. Compared to the Control, rising the temperature from 15 °C to 22 °C had a limited positive effect on decomposition, and for Laceno's soil it was even negative. As for the Water condition, residual weights are always higher than the Control, suggesting an expected slowdown of the decomposition process when the water availability becomes limited and, in accordance to the lower water holding capacity, this phenomenon appears to be more conspicuous for the soil of Pradaccio than from Laceno's.

After 90 days of decomposition, the main factor which remains significant is Condition, which explains 49% of total variance, yet Leaf Material still explains a significant 20% of the variance. The 14 °C drop of temperature from 22 °C to 8 °C did not have a brusque slowdown of the decomposition and the restoration of watering to the soils also did not improve sensibly the decomposition. Likely, a lag-effect between the steps of the experiment is shown here, with the microbial communities needing more time to adapt from a step to another than the experiment's design. However, the soil of Laceno shows a higher resilience than the one from Pradaccio when it comes to drought.

Table 3-3: Results of Three-Way ANOVA for the variable Residual Weight in the three sampling moments of the laboratory decomposition. Values that are statistically significant are bold.

Sampling	Source of Variation	F	*p*
30 days	Leaf Material	0.799	0.377
	Soil	18.984	**<0.001**
	Condition	0.216	0.807
	Leaf Material × Soil	0.043	0.837
	Leaf Material × Condition	0.196	0.823
	Soil × Condition	0.576	0.567
	Leaf Material × Soil × Condition	0.085	0.919
60 days	Leaf Material	19.518	**<0.001**
	Soil	11.264	**0.002**
	Condition	17.679	**<0.001**
	Leaf Material × Soil	3.679	0.063
	Leaf Material × Condition	0.585	0.562
	Soil × Condition	7.338	**0.002**
	Leaf Material × Soil × Condition	1.133	0.333
90 days	Leaf Material	4.342	**0.044**
	Soil	0.655	0.424
	Condition	10.309	**<0.001**
	Leaf Material × Soil	3.328	0.076
	Leaf Material × Condition	0.172	0.843
	Soil × Condition	1.914	0.162
	Leaf Material × Soil × Condition	0.532	0.592

3.2 Enzyme dynamics and relation to mass loss during the on-the-field experiment

All enzymes activities are expressed on the basis of the content of organic matter (Fig. 3-4). The percentage of organic matter on a dry matter basis is similar for both sites, although the graph highlights that, at the first sampling moment, the plant material is the most important factor. Afterwards, the lines diverge according to the location, with the leaves incubated in Pradaccio showing a lesser concentration of organic matter. The same happens when showing the data on the basis of the initial concentration of organic matter related to the weight loss. The higher reduction of organic matter in Laceno is clearly related to the faster decomposition in this site and to the strong increase of some nutrients (e.g. Fe, see further in the text).

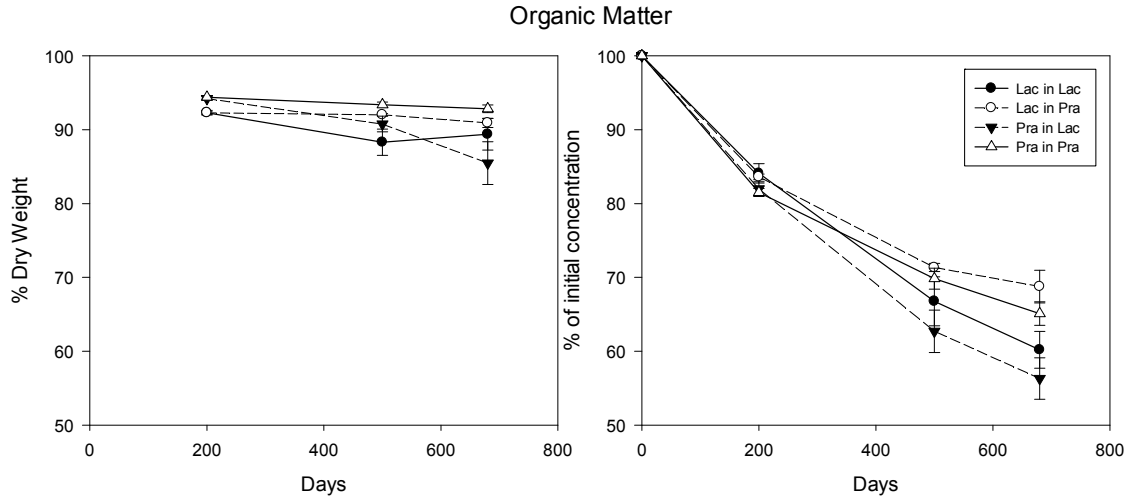


Figure 3-4: Content of organic matter for the on-the-field litterbags expressed as both absolute values and relative values in % of the initial concentration. Values are means±s.e. (n=6).

Among the enzymes analysed, most of them are directly involved in the decomposition processes of holocellulose (cellulase and xylanase) and lignin (laccase and peroxidase). For cellulase and xylanase (Fig. 3-5), the activities are at their highest during the middle point of the decomposition process, while their activity drops in the later stages. Generally, the activity was higher for the soil of Laceno but, within the same soil, Pradaccio's leaves express a higher activity. Laccase and peroxidase (Fig. 3-6), instead, are generally more prominent in the later stages of the decomposition. Mostly, peroxidase activity is much higher than laccase, yet it has to be noted that the activity of laccase is higher in Pradaccio's soil while, for peroxidase, Laceno expresses much higher values.

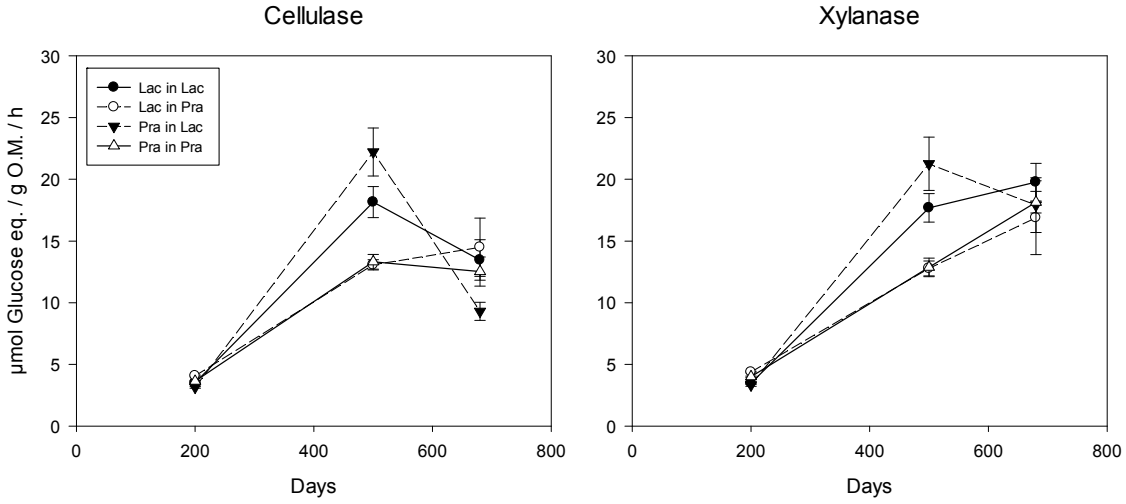


Figure 3-5: Activity of the enzymes cellulase and xylanase in the litterbags during the on-the-field experiment. Values are means±s.e. (n=6).

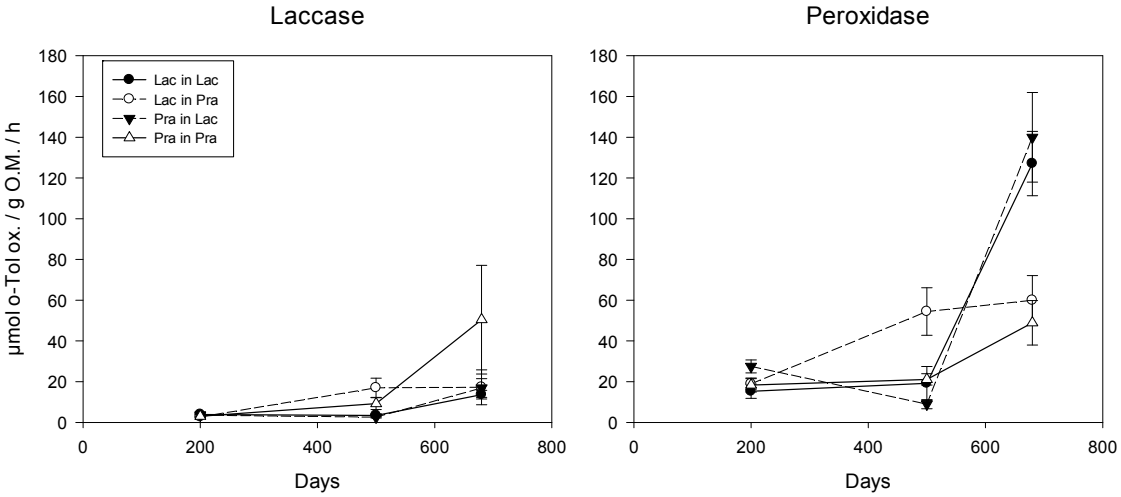


Figure 3-6: Activity of the enzymes laccase and peroxidase in the litterbags during the on-the-field experiment. Values are means±s.e. (n=6).

Among the enzymes related to the cycle of phosphorus, the activities of phosphomonoesterases are shown (both acid and alkaline, Fig. 3-7). As for acid phosphomonoesterase, the trend showed a clear increment during time, which is much more evident for the soil of Pradaccio, regardless of the litter involved. Alkaline phosphomonoesterase displayed a more complex pattern. Initial values were quite different between both Location and Plant Material but, in the middle stage, all values converged to the same point. In the final part of the experiment, some samples showed an increase and others kept on a more or less constant value.

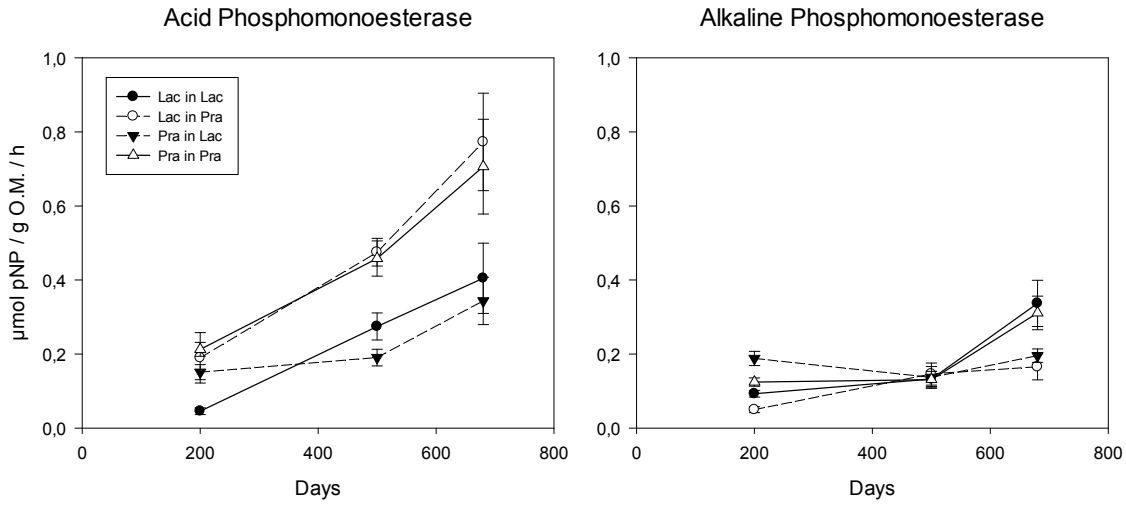


Figure 3-7: Activity of the enzymes phosphomonoesterases in the litterbags during the on-the-field experiment. Values are means±s.e. (n=6).

Among the enzymes that can be more correlated to the cycle of nitrogen, chitinase expressed an activity in continuous growth during the time, generally higher for the site and plant material of Pradaccio (Fig. 3-8). As a measure of total microbial activity, dehydrogenase showed a constant trend for a decrease of activity between the beginning and the middle stage of the experiment, with an increase later on (Fig. 3-8). An exception is *Laceno in Laceno*, where the activity was constant. Generally, it can be appreciated that the overall microbial activity appeared to be higher for the leaves of Pradaccio than for those of Laceno.

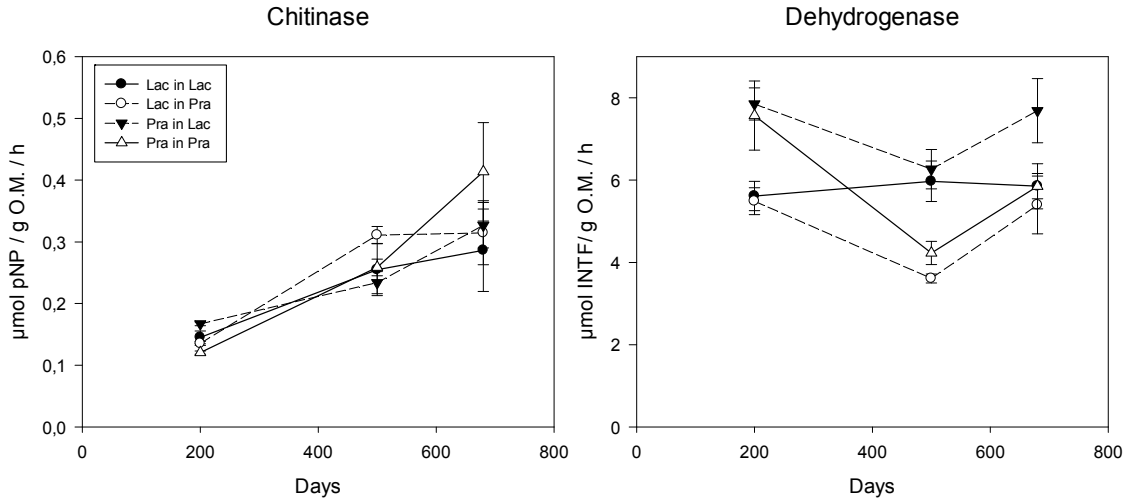


Figure 3-8: Activity of the enzymes chitinase and dehydrogenase in the litterbags during the on-the-field experiment. Please note the differences in the y scale. Values are means±s.e. (n=6).

All the enzymes, with the exception of dehydrogenase, appear to have a very strong correlation with the mass loss (Tab. 3-4). All the Spearman correlation coefficients are negatives, which imply that, the higher the mass loss, the greater the enzymes activities were. The highest correlation coefficient is expressed by xylanase, which can also be used as the single best predictor in a polynomial regression with residual weight as independent variable (Fig. 3-9). The very high R^2(adj.)

(0.748) suggests that, independently of Location or Plant Material, monitoring the activity of an enzyme involved in the degradation of hemicelluloses gives great insight of the proceeding of the decomposition process as a whole.

Table 3-4: Spearman correlation coefficients (r) between the variable Residual Weight and the enzymes activities. Values which are bold are statistically significant.

	r	*p*
Cellulase	-0.658	**<0.001**
Xylanase	-0.817	**<0.001**
Laccase	-0.590	**<0.001**
Peroxidase	-0.493	**<0.001**
Ac. Phosph.	-0.493	**<0.001**
Al. Phosph.	-0.561	**<0.001**
Chitinase	-0.762	**<0.001**
Dehydrogenase	-0,016	0.894

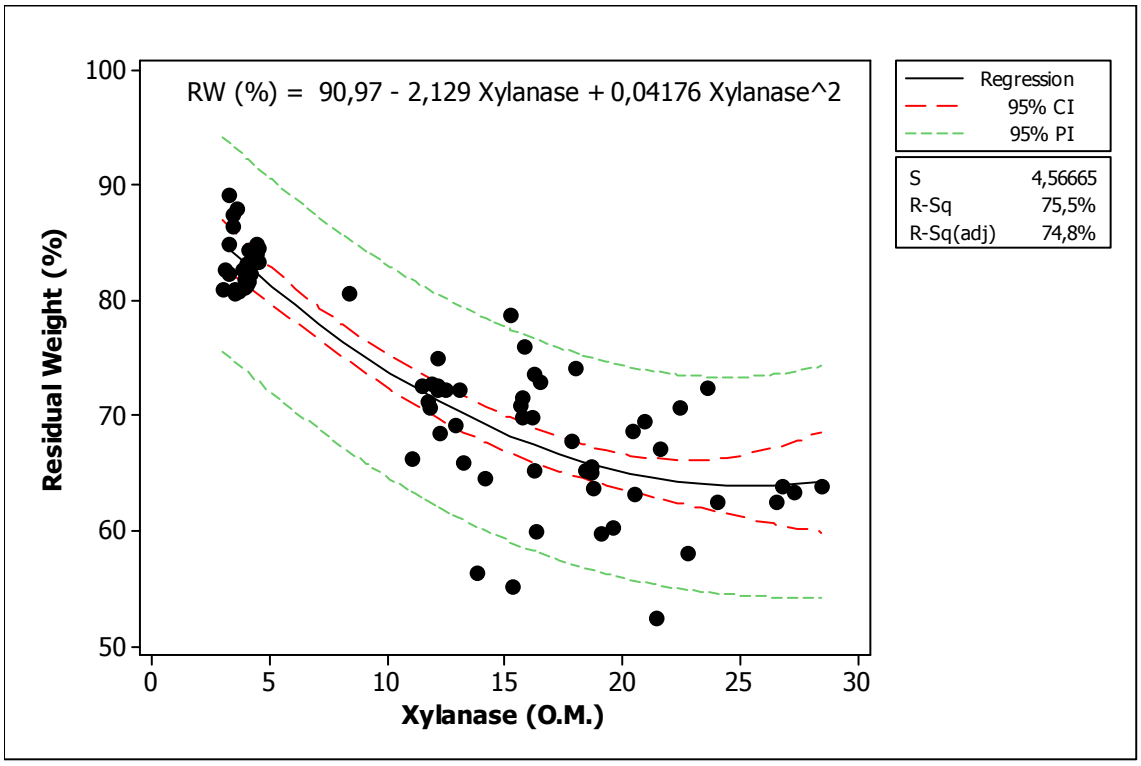


Figure 3-9: Polynomial (2nd order) regression between Residual Weight (dependent variable) and Xylanase (predictor). Red lines represent 95% confidence interval while green lines represent 95% prediction interval.

3.3 Soil chemical features

Nitrogen mineralization was, on the one hand, much higher in Laceno than Pradaccio for NO_3^--N. On the other hand, for NH_4^+-N, the content was higher in Pradaccio than Laceno for the first sampling at 30 days, while after that period the concentration dropped to similar values for both locations (Fig. 3-10). Total nitrogen in the soil was, however, sensibly higher in the northern site than in Laceno (Tab. 3-5).

For the other available nutrients, Pradaccio has a higher availability than Laceno, except for K. In detail, Mg is almost 3 folds higher in Pradaccio, whereas K is 2 folds higher in Laceno. P values are 1.5 fold higher in Pradaccio. Lastly, even though Laceno has a carbonate parent material, the availability of Ca is higher in Pradaccio than Laceno (Tab. 3-5).

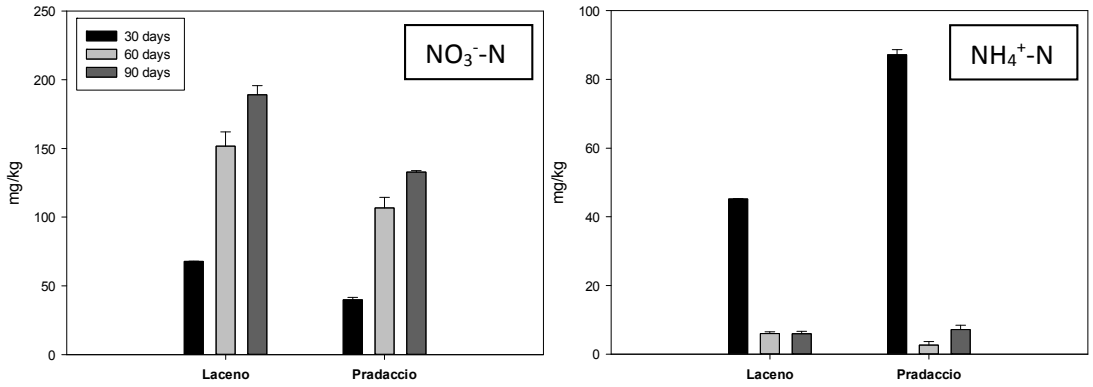


Figure 3-10: Nitrogen mineralization in Laceno and Pradaccio in the three sampling moments for the soil incubation experiment. Values are means±s.e. (n=3).

Table 3-5: Chemical composition of the soils up to 5 cm depth. Values are expressed as mean±s.e. (n=6).

	Laceno	Pradaccio
N tot (mg/kg)	9261±487	11551±1338
P (mg/kg)	8.0±0.2	12.3±0.6
K (mg/kg)	445.0±43.8	213.5±5.8
Ca (mg/kg)	3018.4±23.4	4664.9±86.8
Mg (mg/kg)	287.4±2.0	785.5±15.0
C:N	11.6±0.5	14.3±0.5

3.4 Elements during decomposition

Newly shed litter from Pradaccio and Laceno differed significantly for several elements except S and Fe (Tab. 3-6). Very interesting differences can be appreciated for the macronutrients N (higher in Pradaccio) and P (lower in Pradaccio), whereas for the other macronutrients the largest difference is on Ca (much higher in Laceno) and Mg (lower in Pradaccio). Among the micronutrients, the most interesting difference is Mn (higher in Pradaccio).

Table 3-6: Newly shed litter chemical composition of Laceno and Pradaccio. Values are expressed as mean±s.e. (n=6). Differences are evaluated through a 2-sample T-test and statistically significant ones are bold.

	Laceno	Pradaccio	*p* (same)
N (mg/g)	10.170±0.223	13.056±0.045	**0.006**
P (mg/g)	0.620±0.006	0.404±0.006	**<0.001**
K (mg/g)	1.016±0.013	0.931±0.012	**0.017**
S (mg/g)	2.522±0.556	2.010±0.109	0.462
Ca (mg/g)	15.629±0.041	12.609±0.069	**<0.001**
Mg (mg/g)	1.143±0.016	0.987±0.014	**0.005**
Fe (mg/g)	0.102±0.001	0.101±0.001	0.474
Mn (mg/g)	0.063±0.001	0.140±0.005	**0.004**
C:N	45.9±1.0	36.1±0.1	**0.010**

Table 3-7: Spearman correlation coefficients and significance for nutrients vs. residual weight.

	Coefficient	*p*
N	-0,795	**<0,001**
P	-0,675	**<0,001**
K	-0,464	**<0,001**
S	-0,380	**0,001**
Ca	0,003	0,983
Mg	0,020	0,865
Fe	-0,771	**<0,001**
Mn	-0,719	**<0,001**

A Spearman correlation analysis between the Residual Weight and the concentration of nutrients revealed that some of them (N, Fe and Mn) had a highly negative correlation, while others (P, K and S) had a lower yet significant correlation (Tab. 3-7). Other nutrients (Ca, Mg and Zn) did not show any significant correlation with the mass loss. As concerns the trends of nutrients during the decay of the plant material on the field (Fig 3-11,12,13,14), some nutrients (N, P, Fe and Mn) clearly exhibited a trend of increase during the decay, with Fe increasing its concentration in the leaves up

to 3-4 folds the initial one, especially on the soil of Laceno. Relating to their initial concentration, N and P showed a decrease at the beginning of the experiment and an increase later on. K is known to be easily leached and, although its absolute concentration in the leaves appeared to be slightly increasing in time, related to mass loss its initial concentration dropped to 50-60% of the initial one, displaying a certain variability in the later stages of the experiment. A similar trend is shown for S, even though the concentration of this element dropped substantially for the leaves of Laceno. Ca and Mg have been clearly released from the leaves as they are leached. Mn showed a more leaf-related trend, reaching up to almost 2 folds the initial concentration, especially for the leaves of Laceno decomposing in either location. It has to be highlighted that, for Mn, the concentration of this element in the newly shed leaves of Pradaccio was more than double the one from Laceno, yet for the latter plant material, the data showed a much more sensible immobilization. As for the C:N ratio, a constant linear decrease through time can be observed.

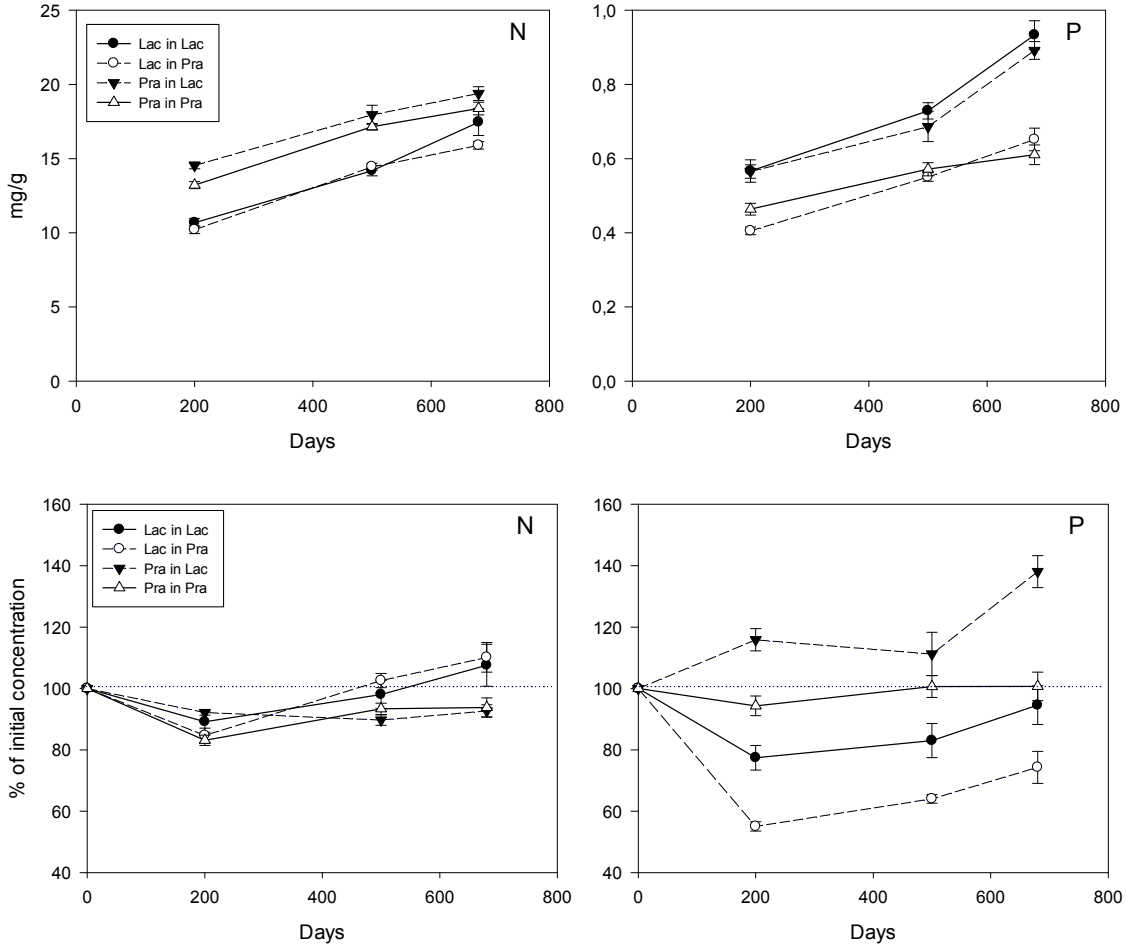


Figure 3-11: Trends of N and P during the on-the-field decomposition expressed as absolute concentration (mg/g) and percentage of the initial concentration. Values are means±s.e. (n=6).

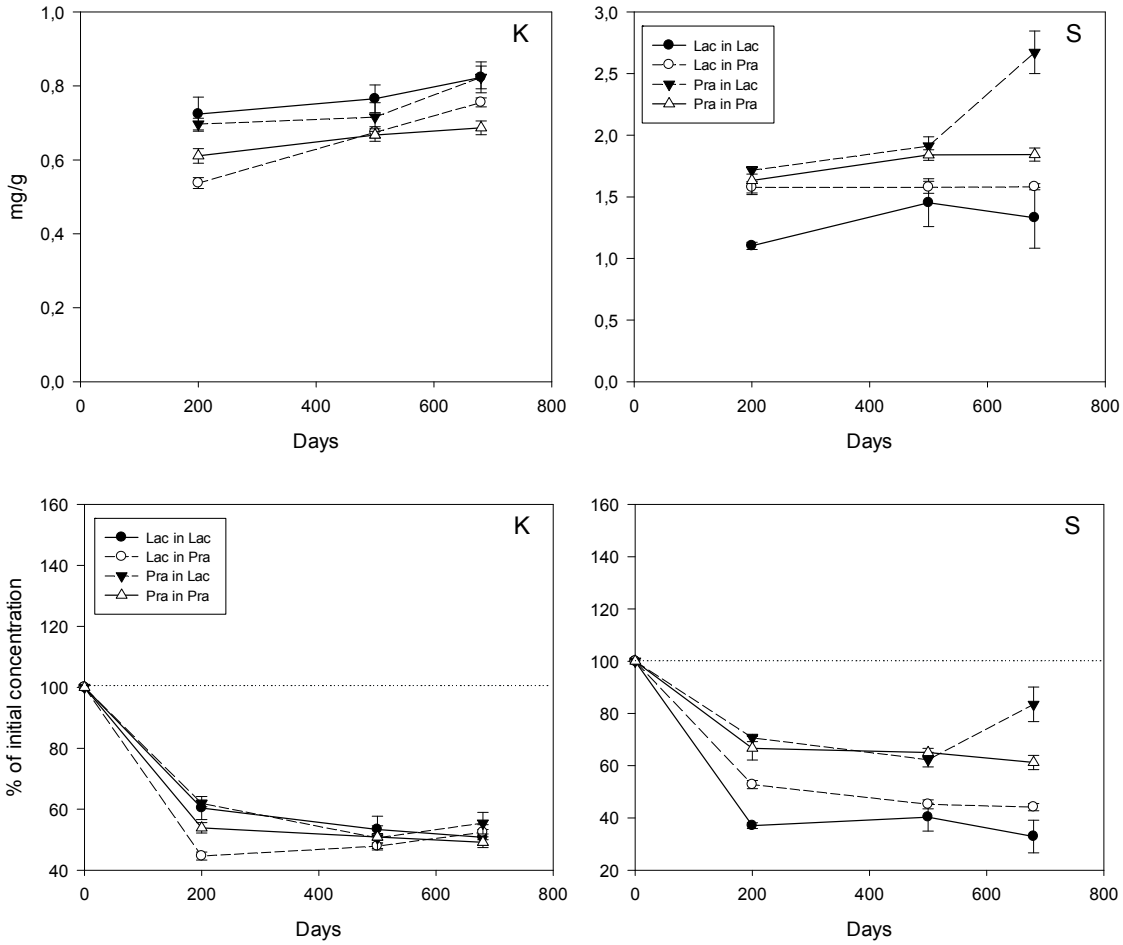


Figure 3-12: Trends of K and S during the on-the-field decomposition expressed as absolute concentration (mg/g) and percentage of the initial concentration. Values are means±s.e. (n=6).

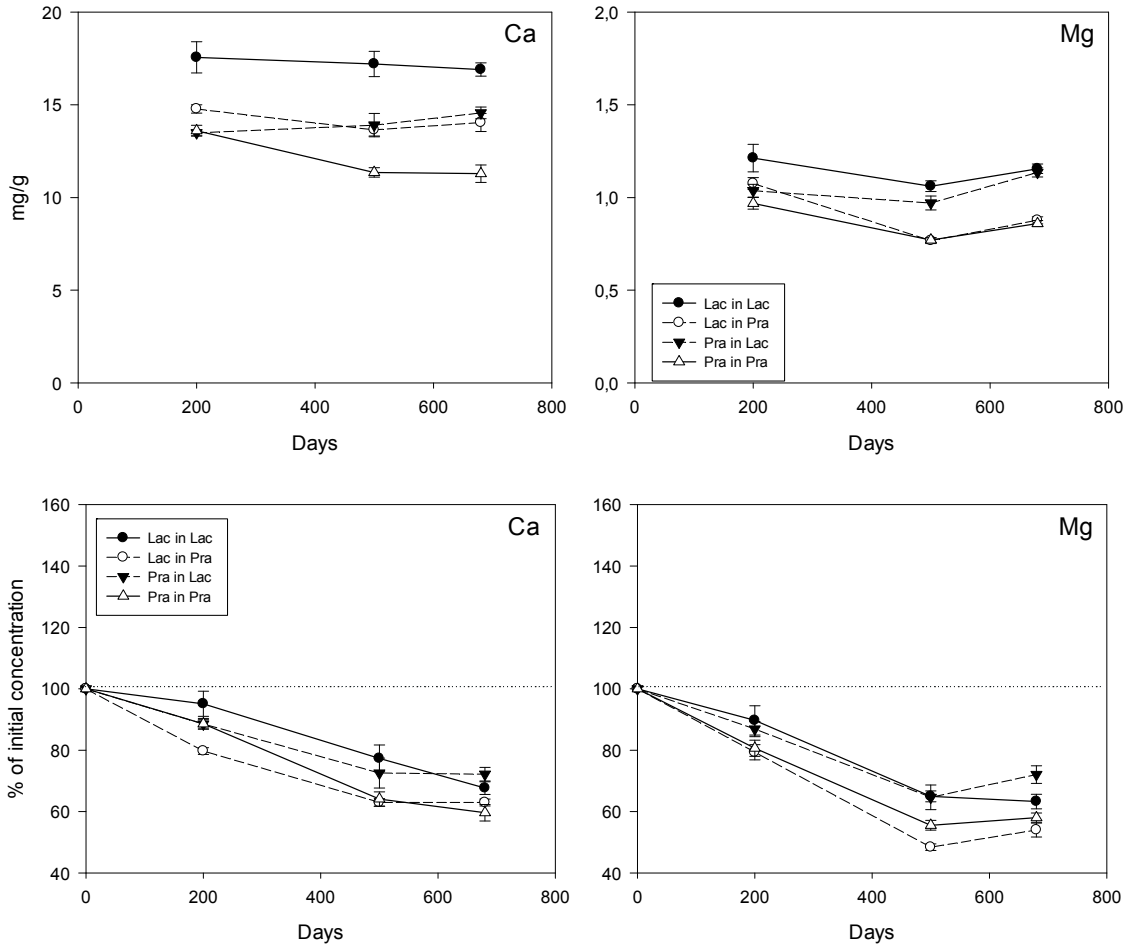


Figure 3-13: Trends of Ca and Mg during the on-the-field decomposition expressed as absolute concentration (mg/g) and percentage of the initial concentration.

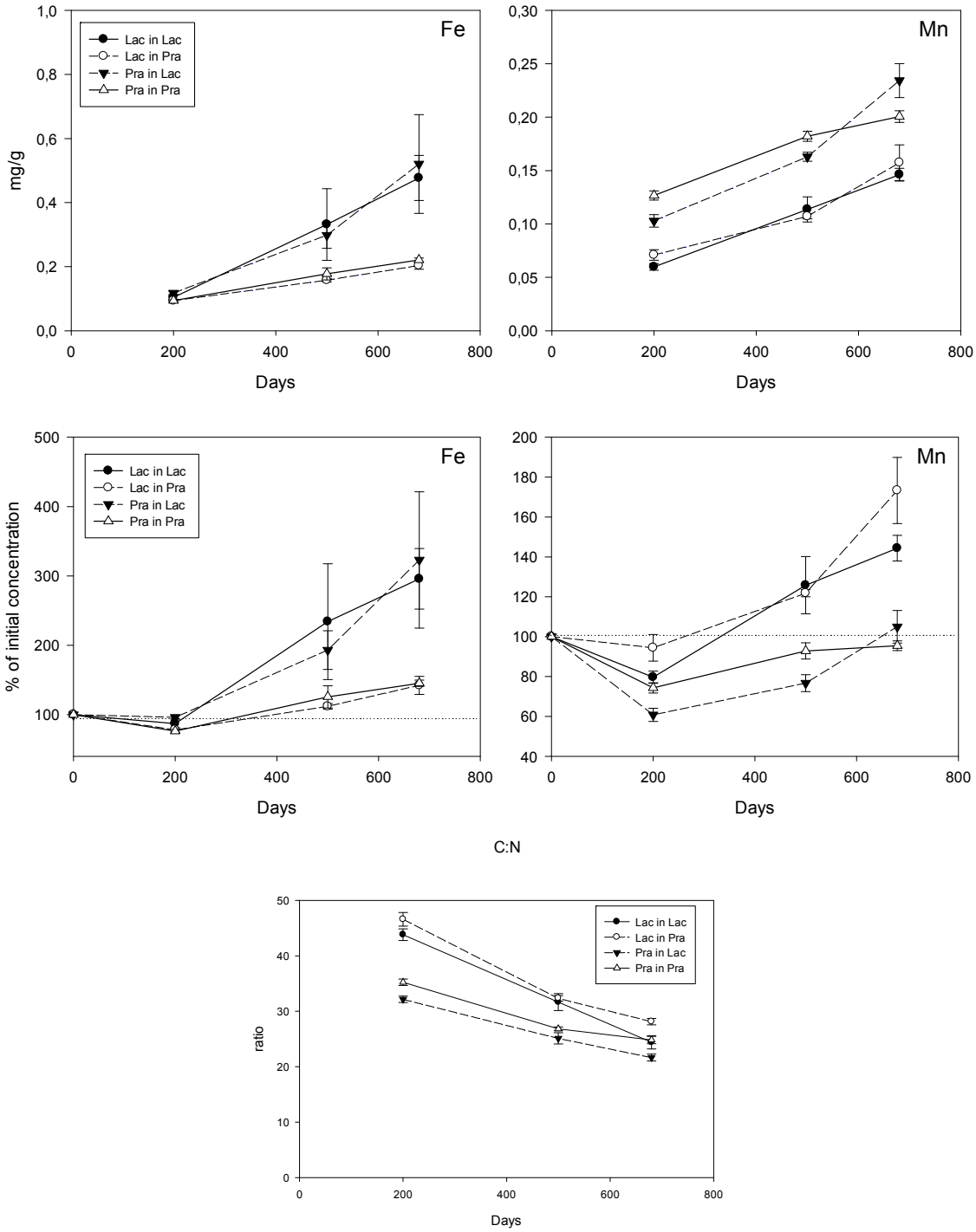


Figure 3-14: Trends of Fe and Mn during the on-the-field decomposition expressed as absolute concentration (mg/g) and percentage of the initial concentration and trends of C:N ratio. Values are mean±s.e. (n=6).

The most important nutrients during decomposition are known to be N and Mn and, on the latter element, great interest has been given to it of late (see Fig. 1-4). Focusing on the effect of Plant Material and Location for N and Mn during the three samplings (Tab. 3-8), it emerges that, in the initial stages, both Plant Material and Soil are significant in the variance of the elements, even though the difference justified by the different initial plant material is always much more relevant. At 500

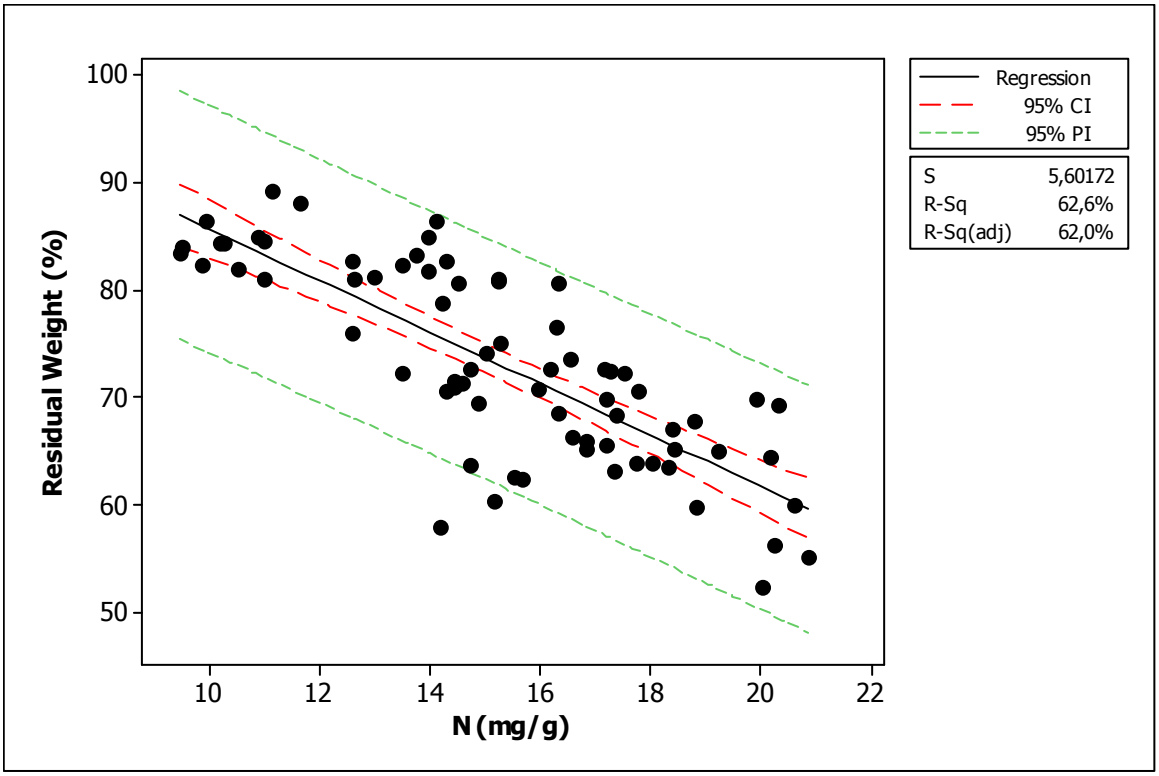
and 680 days, for both N and Mn the variance is explained in a significant way only from the different Plant Material, although for N at 680 days the Location factor becomes significant again.

Table 3-8: Two-Way ANOVA results for N and Mn for the three sampling moments of the on-the-field experiment. Statistically significant values are bold.

		N		Mn	
Sampling	Source of Variation	F	*p*	F	*p*
200 days	Plant Material	179,5	**<0,001**	112,5	**<0,001**
	Location	12,3	**0,002**	14,1	**0,001**
	Plant Material × Location	2,8	0,110	1,8	0,192
500 days	Plant Material	65,8	**<0,001**	85,2	**<0,001**
	Location	0,5	0,500	0,9	0,357
	Plant Material × Location	1,8	0,191	3,6	0,072
680 days	Plant Material	15,2	**0,001**	28,9	**<0,001**
	Location	5,2	**0,034**	0,9	0,366
	Plant Material × Location	0,2	0,627	3,4	0,080

N can be used a predictor or Residual Weight with a simple linear regression line with great accuracy (R^2adj.=0.620) but also Mn is shown to have great predictability of the decomposition process through a quadratic regression, although with a lesser variability predicted (R^2adj.=0.515) (Fig. 3-15). Combining both elements together in a multiple linear regression, the predictability improves only slightly compared to N alone (R^2adj.=0.625 vs. 0.620) and this is due to the fact that the relationship between Mn and Residual Weight is not linear.

$$RW(\%) = 107.4 - 2 \cdot N - 27 \cdot Mn \; [S=5.57;\; R^2=0.635;\; R^2adj.=0.625]$$



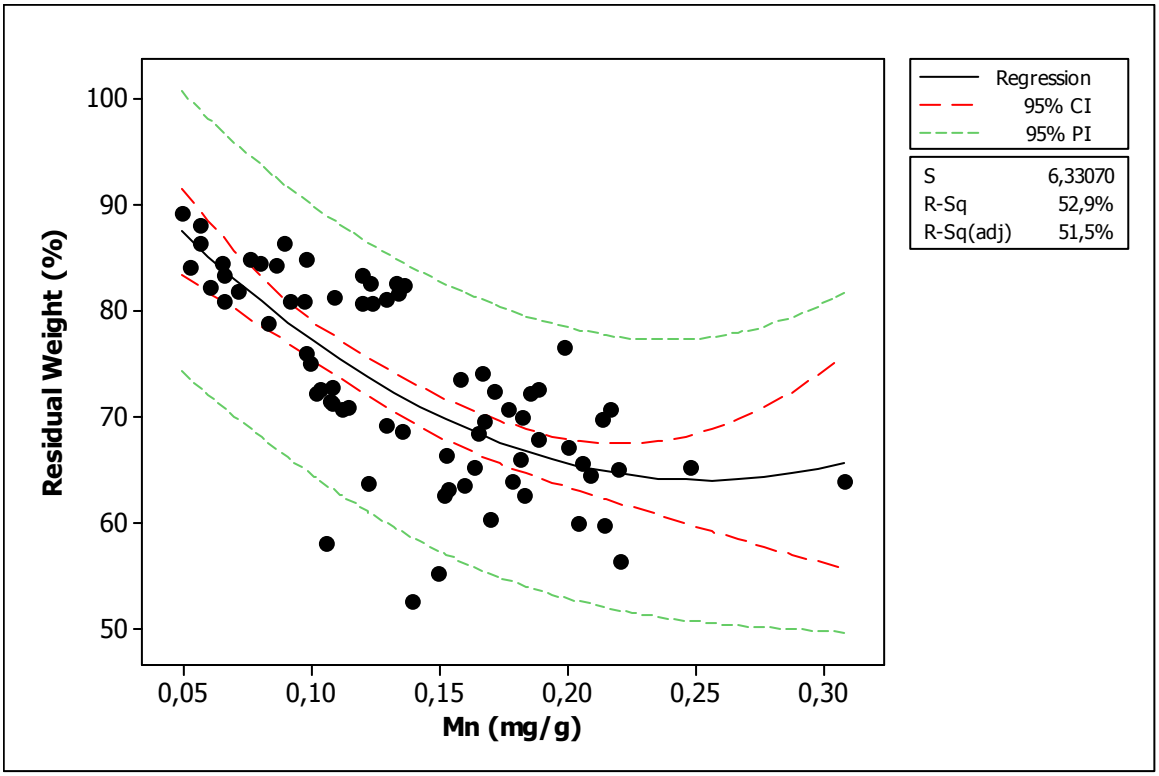


Figure 3-15: Linear regression between Residual Weight (dependent variable) and N (predictor) shown above and Polynomial (2nd order) regression between Residual Weight (dependent variable) and Mn (predictor) shown below. Red lines represent 95% confidence interval, while green lines represent 95% prediction interval.

Equations are $RW=109.6-2.3 \cdot N$ *and* $RW=100-287 \cdot Mn+567 \cdot Mn^2$

In laboratory conditions, the results from the nutrients' concentration reflect the complexity of the patterns of residual weights in different conditions. Looking at correlations (Tab. 3-9), the only nutrient which has always a significant negative correlation with residual weight is N, although the correlation appears to be weaker than the field condition. Also in contrast to the field, P is positively correlated to the residual weight, even though in a significant way only for the Water condition. Ca confirms its lack of connection to the residual weight, while K and S have a more unstable correlation to the loss of weight in the three conditions. Fe confirms its strong link to the residual weight only in the Temperature condition. Mg, which proved to be uncorrelated in the field experiment, shows a significant negative correlation especially, once again, in the Temperature treatment. The only variable which, as N, has a significant and almost constant correlation with the residual weight (although not in a significant way for the Water treatment) is Mn. For reasons simplicity, not show all the graphs for the trends of all the nutrients are shown, but focus will be given only on some specific interesting nutrients, namely N and Mn.

Table 3-9: Spearman correlation coefficients and significance for nutrients vs. residual weight in the three laboratory conditions.

	CONTROL		TEMPERATURE		WATER	
	Coefficient	*p*	Coefficient	*p*	Coefficient	*p*
N	-0.340	**0.018**	-0.395	**0.006**	-0.316	**0.029**
P	0.236	0.106	0.271	0.063	0.443	**0.002**
K	-0.166	0.258	0.323	**0.025**	0.151	0.305
S	-0.028	0.847	0.394	**0.006**	0.325	**0.024**
Ca	-0.173	0.238	-0.003	0.986	0.134	0.363
Fe	-0.260	0.074	-0.669	**<0.001**	0.013	0.929
Mg	-0.570	**<0.001**	-0.628	**<0.001**	-0.135	0.357
Mn	-0.501	**<0.001**	-0.510	**<0.001**	-0.262	0.072
Zn	0.266	0.068	-0.137	0.352	0.364	**0.011**

The trends for N (Fig. 3-16) show a tendency to increase in their absolute concentration, but it is more interesting to observe the trends related to their initial concentration. In the control condition, it is shown a release of N of proximately 20%. This is in accordance to the field data, where at the first sampling moment (200 d that correspond to proximately 90 d in microcosms) a release of N before its immobilizing is shown. In the Temperature treatment, the trend is comparable to the control, except for Laceno in Pradaccio where, at the last sampling moment, N is immobilized. In the Water condition, a tendency to increase in the last sampling moment is shown for all samples.

Looking at the trends for Mn during the laboratory conditions (Fig. 3-17), we have a strong similarity to what happens in the field. It can be noted that, as with the field data, the leaves with a higher initial Mn content (Pradaccio) decompose faster and, when decomposition slows down (i.e. in Water treatment) the increase in Mn slows down as well. Finally, the most important point is the

neat higher increase of Mn from its initial concentration in those leaves which were poorer for this element at the beginning (Laceno).

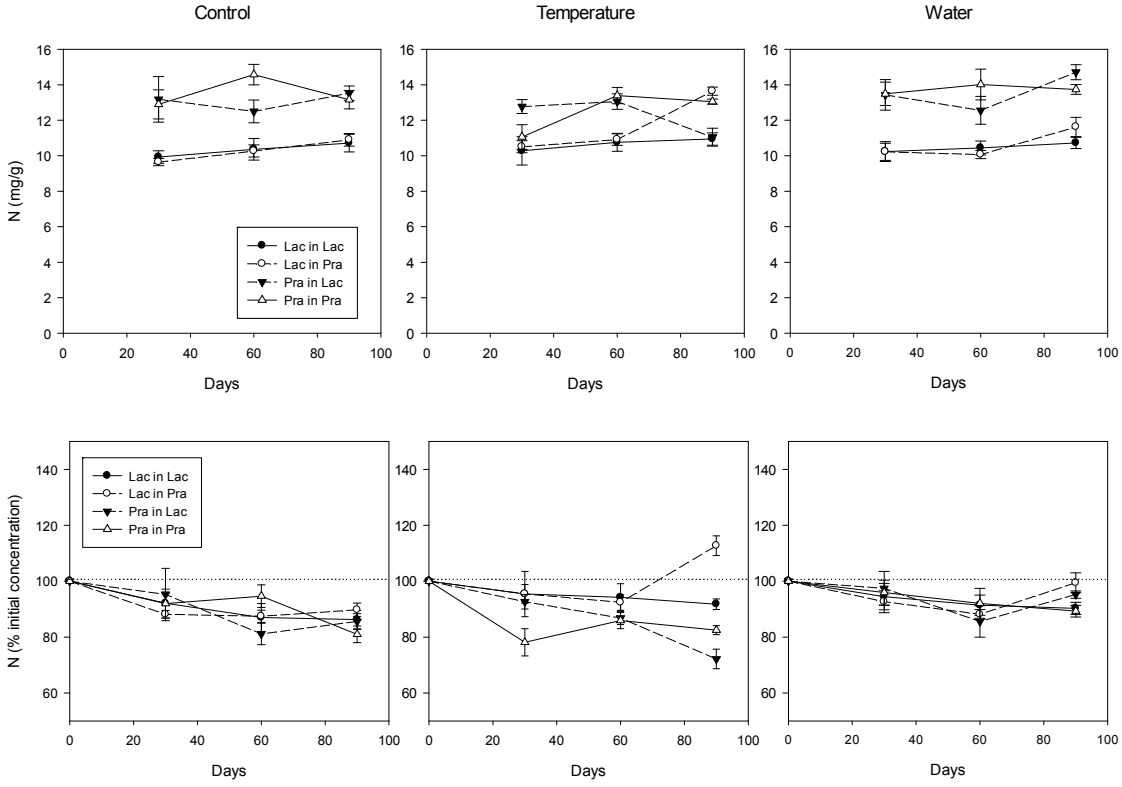


Figure 3-16: Trends of N during the three laboratory conditions expressed as absolute concentration (mg/g) and percentage of the initial concentration. Values are mean±s.e. (n=4).

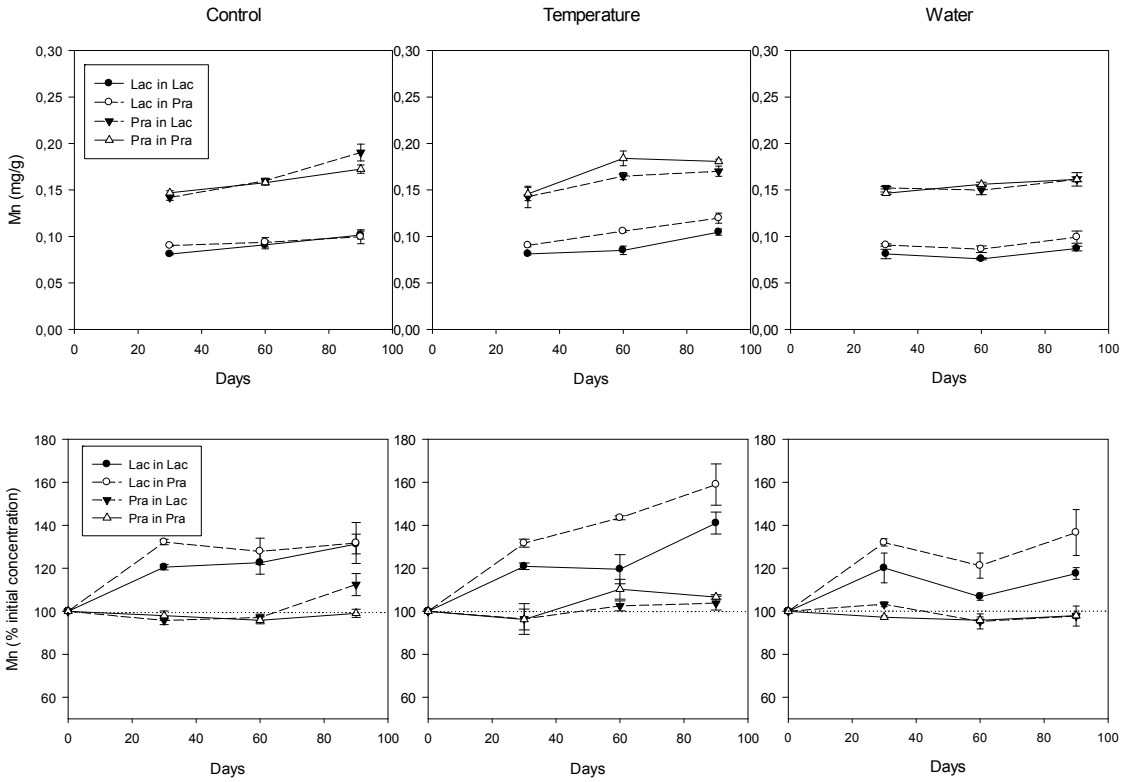


Figure 3-17: Trends of Mn during the three laboratory conditions expressed as absolute concentration (mg/g) and percentage of the initial concentration. Values are mean±s.e. (n=4).

The correlation between Mn and Residual Weight at the very beginning of the decomposition process (which is the case of the litterbags microcosm experiments) is shown to be linear and not quadratic at least in the Control condition. Comparing two linear regression analysis with two subsets from the leaves of Laceno and Pradaccio in the Control condition, it is observable that, even though the absolute values of Mn are lower in Laceno, its increase related to the Residual Weight is much faster than what happens in Pradaccio (Fig. 3-18).

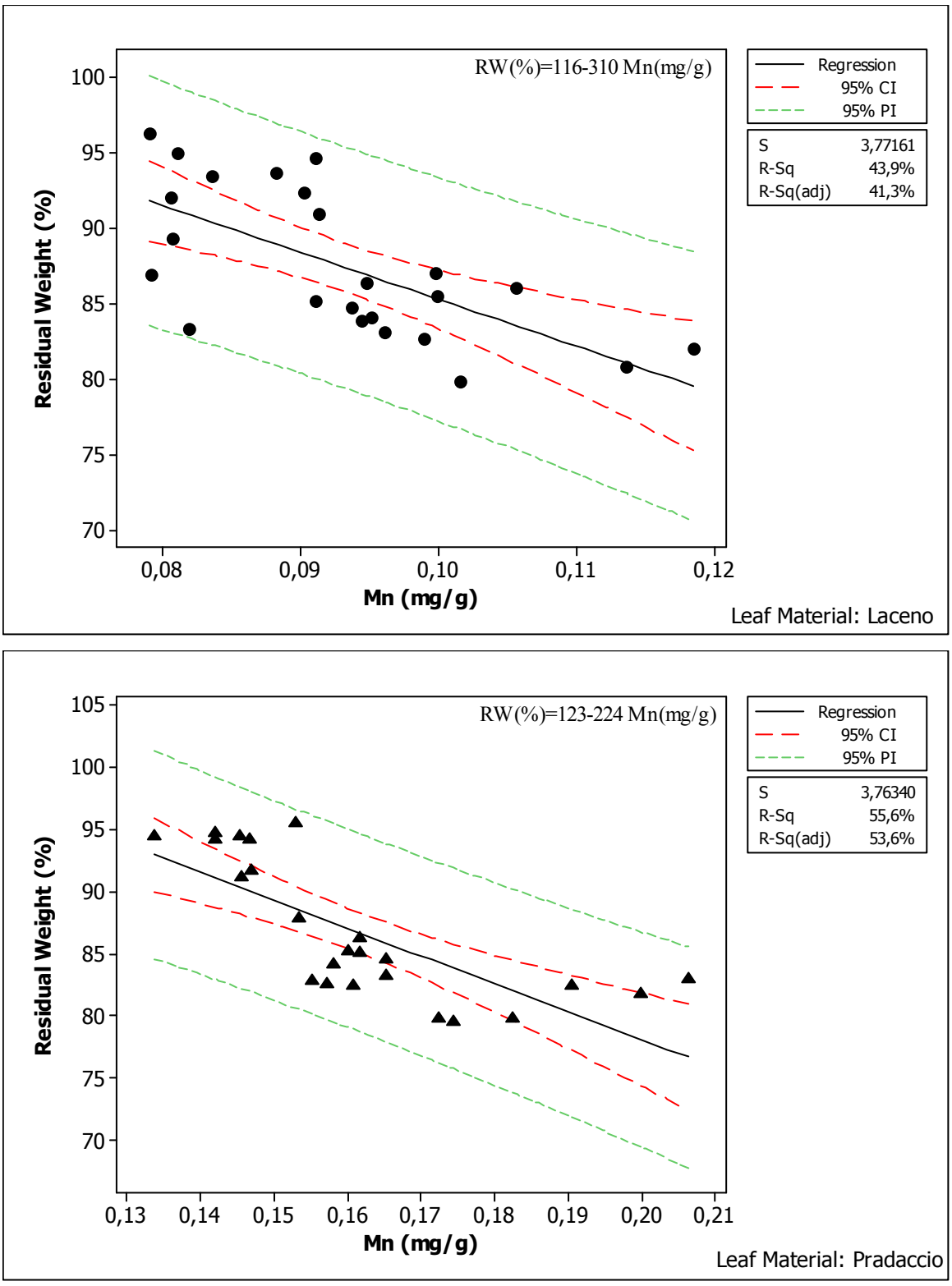


Figure 3-18: Regression equations for the subsets of the two different Leaf Material with Residual Weight as dependent variable and Mn as predictor. Only data from the Control condition are shown. Red lines represent 95% confidence interval, while green lines represent 95% prediction interval.

As for the field data, to assess when and how the variance happens according to different factors, a Three-Way ANOVA has been performed on N and Mn in the three samplings of the laboratory experiment (Tab. 3-10). Once again, strong similarities with the field data can be appreciated, especially for Mn. Moreover, on the variable Residual Weight under laboratory condition the results from the Three-Way ANOVA match almost completely the ones for Mn, highlighting once again the strong connection between Mn and mass loss.

Table 3-10: Three-Way ANOVA on N and Mn in the laboratory conditions.

		N		***Mn***	
Sampling	Source of Variation	F	*p*	F	*p*
30 days	Leaf Material	45.705	**<0.001**	551.741	**<0.001**
	Soil	0.715	0.403	3.775	0.060
	Condition	1.047	0.362	0.486	0.619
	Leaf Material × Soil	0.629	0.433	2.796	0.103
	Leaf Material × Condition	2.104	0.137	0.415	0.664
	Soil × Condition	0.317	0.730	0.398	0.675
	Leaf Material × Soil × Condition	0.703	0.502	0.488	0.618
60 days	Leaf Material	83.726	**<0.001**	974.013	**<0.001**
	Soil	3.553	0.068	16.401	**<0.001**
	Condition	0.229	0.796	19.780	**<0.001**
	Leaf Material × Soil	4.886	**0.034**	0.537	0.468
	Leaf Material × Condition	0.640	0.533	2.433	0.102
	Soil × Condition	0.477	0.625	5.961	**0.006**
	Leaf Material × Soil × Condition	0.947	0.397	0.044	0.957
90 days	Leaf Material	59.777	**<0.001**	502.056	**<0.001**
	Soil	10.362	**0.003**	0.897	0.350
	Condition	2.842	0.071	10.452	**<0.001**
	Leaf Material × Soil	5.095	**0.030**	2.972	0.093
	Leaf Material × Condition	19.709	**<0.001**	2.686	0.082
	Soil × Condition	12.174	**<0.001**	4.537	**0.017**
	Leaf Material × Soil × Condition	0.822	0.448	0.296	0.745

3.5 Discussion

3.5.1 Effects of plant material and climate on mass loss

Table 3-11: Comparison of this study (bold) with other similar studies on beech leaf litter decomposition. References are D'Annunzio et al., 2008 (1); Albers et al., 2004 (2); Sariyildiz and Anderson, 2003 (3) Kooijman and Martinez-Hernandez, 2009 (4); this Ph.D. thesis (5); Cortez, 1998 (6); Rutigliano et al., 1989 (7).

Reference	Location	Nation	Lat	Long	Elevation (m amsl)	Average T (°C)	Average Rainfall (mm)	Soil pH (H_2O)	Tree Average Age (y)	T 50% (days)	k (d^{-1})
1	Sorø	Denmark	55.48	11.63	466	8.0	510	4.4	84	990	7.00^{-4}
2	Solling	Germany	51.72	9.62	410	8.2	1300	3.5	30	990	7.00^{-4}
1	Leinefelde (L2)	Germany	51.08	10.47	445	7.0	750	4.5	62	693	1.00^{-3}
3	Devon	U.K.	50.62	-3.80	260	10.5	1100	4.3	N.A.	866	8.00^{-4}
4	Diekirch	Luxemburg	49.85	6.15	220	9.0	789	5.5	150	495	1.40^{-3}
1	Fougères (F5)	France	48.38	1.15	190	11.0	890	3.9	80	866	8.00^{-4}
1	Charmois-l'Orgueilleux (Het88)	France	48.10	6.23	400	5.0	1100	3.9	68	577	1.20^{-3}
5	**Pradaccio**	**Italy**	**44.53**	**10.02**	**1355**	**6.0**	**2600**	**4.1**	**75**	**1155**	$\mathbf{6.00^{-4}}$
6	Saldès	France	44.10	3.37	860	8.1	2355	4.8	N.A.	1035	6.70^{-4}
7	Monte Taburno	Italy	41.05	12.07	1100	7.9	2166	6.0	100	803	8.63^{-4}
5	**Laceno**	**Italy**	**40.78**	**15.08**	**1176**	**8.6**	**2400**	**5.7**	**75**	**990**	$\mathbf{7.00^{-4}}$

The decomposition trends in the field experiment offer results that are comparable with similar researches on the same plant material (Tab. 3-11). The most comparable results, both for decomposition coefficient and ecological characteristics, are the ones from the Saldès hill in the Cévennes National Park in France (Cortez, 1998). The results are also similar from Monte Taburno, a beech stand not very far away from Laceno on another mountain complex of the Apennines. In this mountains the decomposition has been recorded to be slightly faster than Laceno (Rutigliano et al., 1989), even though the environmental factors are rather similar.

As for continental and oceanic climates of Middle Europe, particularly similar are Sorø, Denmark (D'Annunzio et al., 2008) and Solling, Germany (Albers et al., 2004) but differences emerge with other studies, where the decomposition trends were usually faster. A clear and unambiguous pattern of decomposition in the beech forests of Europe cannot be assessed but, on average, the decomposition on the Italian Apennines appear to be slower than most of the cases in Middle Europe. Several variables, including the different topography of mountain beech forests of the Apennines, different vegetation history and other edaphic features are required to assess, particularly the chemical composition of both soil and plant material.

From the microcosm experiment, the results from the Control condition are comparable with a similar research which also run for a maximum of 90 days (Cortez et al., 1996) and with a recent experiment on early step of decomposition in beech which ran for 8 weeks (Brandstätter et al., 2013) even though with some methodological differences in both cases.

The results suggest that, in terms of loss of weight, on the field plant quality is a driving factor for the first phase of the decomposition (200 days), while the different locations increase its relevance in the later stages (200 to 680 days). This results are conspicuous to some general assessments of the decomposition process (Berg and McClaugherty, 2014). Under laboratory condition, where the focus is more only on the very early stages of decomposition, we showed a higher impact of the soil type at the beginning of the experiment (first 30 days), whereas plant material becomes significant later on (60 to 90 days) and multifarious interaction between plant material, soil and climatic conditions are triggered, suggesting a complex microbial response to the different factors. Accordingly, other experiments in microcosm, comparing freshly shed litter and litter already in a more advanced stage of decomposition, showed that a significant effect of plant quality has been noted only in the second case (Cortez et al., 1996). Similarly, over short period of incubation (8 weeks), Brandstätter et al. (2013) suggest that the factor Time was more predictive than the factor Location, indicating that main modifications occurred during the initial stages of decomposition.

The comparison between the results produced by my experiment on the field and the ones gained under laboratory condition put forward that, even though on the field the leaves decaying in the forest of Pradaccio have a smaller decomposition constant, when this soil and its microbial communities undergo favourable constant conditions of temperature and humidity the decomposition on the soil of Pradaccio is faster, at least for the early stages of decomposition.

The forest of Laceno has temperatures that, during summer, can easily go above 22 °C, whereas in Pradaccio this is much rarer. Thus, we can state that Laceno has a more Mediterranean-oriented climate in comparison to Pradaccio, and it has been proved that hot and dry weather during summer can lead to a sensible reduction of the microbial activity (Fioretto et al., 2005) while the more favourable conditions of spring and autumn can increase the decomposition rate (Coûteaux et al., 1995). Moreover, biological activity is usually higher in calcareous soils than acidic ones (Ponge, 2003). The different behaviour of the two microbial communities in the soils suggest that, for a colder site, higher temperatures are a favourable condition, whereas for a hotter site they slow down decomposition because, in terms of adaptation of the soil biota, higher temperatures are related to aridity and unless there is enough moisture, usually more than 10% water-holding capacity, water may be so limiting that no higher microbial activity results from any temperature increase (Berg and McClaugherty, 2014). Likely, the soil biota in Laceno is adapted to good functionality for longer periods during spring, autumn and parts of winter since they are hindered by the semiarid and warm summer.

On the contrary, in Pradaccio, where the temperatures are much more rigid, the snow cover is far more abundant and the summer drought is not so stressed, the microbial communities are expected to be more active in late spring and summer. As a matter of fact, in cold climate ecosystems, microorganisms would be adapted to the prevalent climatic conditions (Berg and McClaugherty, 2014). All of this is confirmed by the higher decomposition rate for the leaves decomposing on the

soil of Pradaccio where the temperature is shifted from 15 °C to 22 °C, but also from the more sensibility of this soil to drought compared to the one in Laceno. In terms of climate change, these phenomena should be taken into account in order to consider the potential shift of the decomposition patterns and nutrients cycle not only in stable forms of C in the soil (Meier and Leuschner, 2010) but also for early stages of decomposition. Additionally, given that our mesh size of our litterbags was large enough to allow an impact of part of mesofauna, the role of earthworms, especially for the soil of Pradaccio in the microcosms, cannot be fully excluded, as they have been already shown to favour the decomposition of litter material also for beech (Cortez and Bouché, 2001, 1998).

3.5.2 Enzyme dynamics

Generally, the results demonstrate that all the enzymes sampled in the on-the-field experiment (except dehydrogenase) show a highly significant negative correlation with the mass loss. This implies that a careful analysis of the different trends can give a good insight at what is happening and which compartment of the microbial community is likely involved.

As for the activity of dehydrogenase, this enzyme is, as mentioned is a measure of total microbial activity, and it is usually measured in the soil rather than in the litter (Quilchano and Marañón, 2002; Von Mersi and Schinner, 1991). Likely its importance is higher in the soil than in the litter, where C and N become much more limiting, especially with depth, whereas in the litter it just shows a very high microbial activity but does not allow to discriminate between the microbial communities. The only input that we can collect from this enzyme is that the soil biota appears to be more active in Pradaccio than Laceno.

A complex picture is formed by the data coming from the enzymes involved in the degradation of holocellulose (cellulase and xylanase) and lignin (laccase and peroxidase). It is worth to remind that, although cellulose is a more easily degraded polymer, most of it is physically protected by lignin in the cell walls, thus neither group of compounds decompose independently (Cooke and Whipps, 1993). This study confirms that the degradation of lignin and cellulose is very slow at the beginning but, after some time, the activities of cellulase and xylanase greatly increase. At the same time, the activities of laccase and peroxidase slowly increase and they reach their maximum activity once the cellulase and xylanase slow down. Clearly, the decomposition of holocellulose starts only when peroxidase and laccase begin the aggression of lignin, exposing the holocellulose. Once the holocellulose is decreasing, the activity of laccase and peroxidase strongly increase, as lignin starts to become the most abundant plant tissue remaining. Similar trends, although in different conditions and plant species, have already been reported (Fioretto et al., 2000; Papa et al., 2008). As for the differences between Laceno and Pradaccio, cellulase and xylanase are shown to be more active in the southern location and this is in accordance to the higher decomposition trend recorded in Laceno. Laccase and peroxidase both contribute to the degradation of lignin, but they are involved in different

parts of the process and secreted by different fungi. Usually, peroxidase begins the degradation of lignin and laccase is involved in the last parts of it (Osono, 2007). The shifting between these two enzymes in Laceno and Pradaccio at the last sampling moment (laccase higher in Laceno and peroxidase higher in Pradaccio) are connected to the faster decomposition happening in Laceno and to the higher availability of Mn in Pradaccio. The correlation between peroxidase and Mn can be appraised by a linear regression of the activity of peroxidase and the increase of Mn during decomposition. Although the R^2(adj.) is quite low (0.145), the model is highly significant (p=0.001), strengthening the knowledge of the connection between Mn and peroxidases (Fig. 3-19).

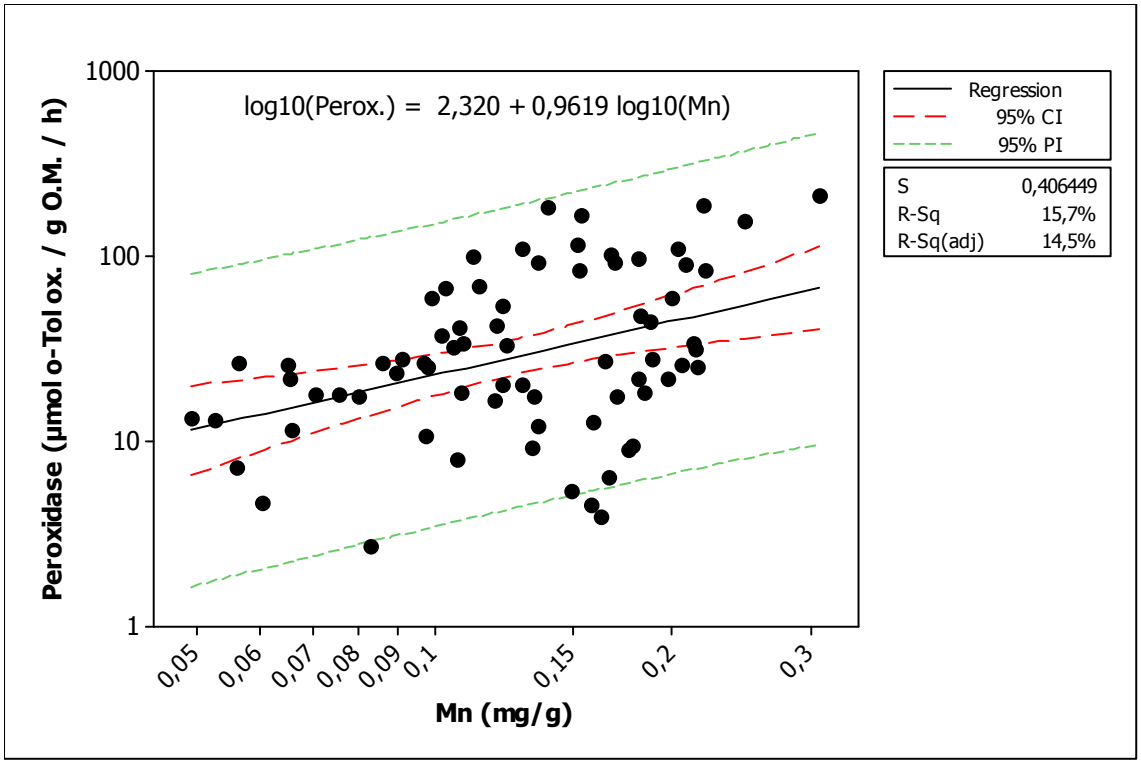


Figure 3-19: Linear regression (on log10 transformed data) using Peroxidase as dependent variable and Mn (expressed as mg/g) as predictor. Red lines represent 95% confidence interval, while green lines represent 95% prediction interval.

A trend similar to laccase is shown by chitinase that are higher in Pradaccio than Laceno. Chitinase degrade chitin, which is, as known, the major constituent of fungi's cell wall. Thus, monitoring the increase of chitinase is an indirect way to monitor the growth of the fungal community, which is highly dependent on the N availability (Fig. 3-20). The pattern is similar with acid phosphomonoesterase, although there is not a very strong direct correlation with the availability of P (data not shown).

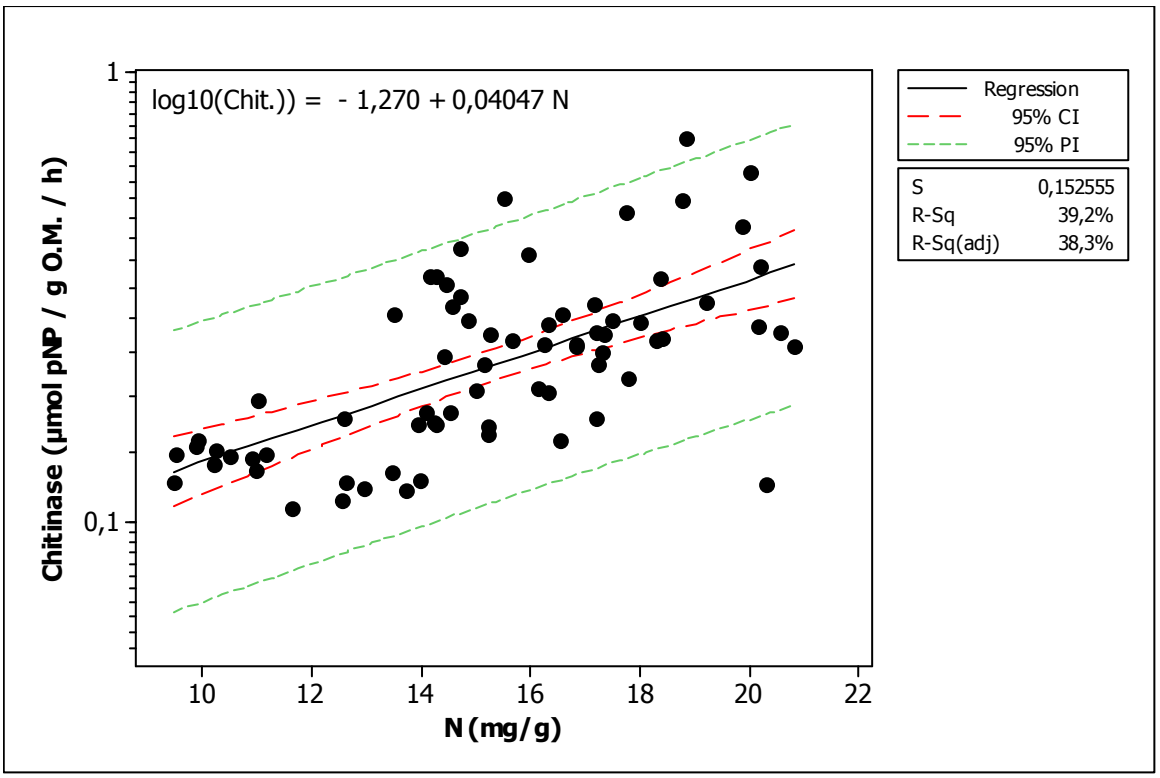


Figure 3-20: Linear regression using Chitinase (on log10 transformed data) as dependent variable and N (expressed as mg/g) as predictor. Red lines represent 95% confidence interval, while green lines represent 95% prediction interval.

Although the general pattern of the enzymes dynamics is similar to those shown in other studies involving plant litter from European forests (e.g. Kjøller and Struwe, 2002; Šnajdr et al., 2008), few studies simultaneously dealt with decomposition in litterbags and enzymes dynamics (Andersson et al., 2004; Di Nardo et al., 2004; Fioretto et al., 2000; Papa et al., 2008).

Although not currently considered in this project, the identification of the microbial communities during decomposition through molecular procedures is a great asset in understanding the precise role of fungi and bacteria during decomposition (e.g. Agnelli et al., 2004; Ibekwe and Grieve, 2004; König et al., 2010; Schneider et al., 2012; Watanabe et al., 2004). Nevertheless, enzymes greatly contribute to understanding the proceeding of microbial communities and give great insight to the functional role of fungal succession in a quick, easy and not expensive way (Baldrian, 2009; Burns et al., 2013; Nannipieri, 2006; Nannipieri et al., 2012).

3.5.3 Soil and litter chemical properties

Focusing on chemical quality, the two forests have more or less the same age, therefore forest age cannot be considered as a factor involved (Trap et al., 2013). Nevertheless, great differences emerge between the quality of the plant material, even though this has already been proved to be particularly true for beech trees (Brandstätter et al., 2013; Sariyildiz and Anderson, 2003; Sariyildiz et al., 2005).

The chemical composition of our leaves is in range with those measured in other studies for European beech (Brandstätter et al., 2013; Joergensen et al., 2009; Sariyildiz and Anderson, 2005; Vesterdal, 1999) even though differences are conspicuous especially for Mn. A contrasting feature is the higher concentration of available Ca in Pradaccio's soil, whereas, as expected, this nutrient is more abundant in Laceno's plants, which grow on calcareous soil. This discrepancy is likely due to the extraction with neutral NH_4OAc which can lead to an overestimate of cations under severely acidic pH, as is the soil from Pradaccio (Ross and Ketterings, 1995).

According to Vesterdal (1999), beech litter mass loss was positively affected by nutrient status of the soil and that soil types influence decomposition in different ways and to a different extent, especially where there is higher quality litter. The presented research agrees with the latter assessment but, in contrast to the above-mentioned study, the locations that have been investigated have sensible climatic differences. As a matter of fact, although, on a general line, both the soil and litter quality appear to be higher in Pradaccio, on the field the decomposition is higher in Laceno, while in laboratory conditions decomposition proceeds faster for both plant and soil from Pradaccio.

As for the role and mobilization of nutrients during the decay, the strong increase of Fe could be related to earthworms activities which deposit thin particles of soil on the plant material with their faeces (Cortez and Bouché, 1998) and a significant increase of Fe compared to all other elements has been proved when mesh size is 1 mm^2 (Joergensen et al., 2009). Other nutrients (K, P, Ca, Mg) have trends which are comparable to the available literature (Berg and Laskowski, 2006; Berg and McClaugherty, 2014), with usually a decline in concentration over the first decomposition period followed by a more or less continuous increase until the end of decomposition period (Joergensen et al., 2009).

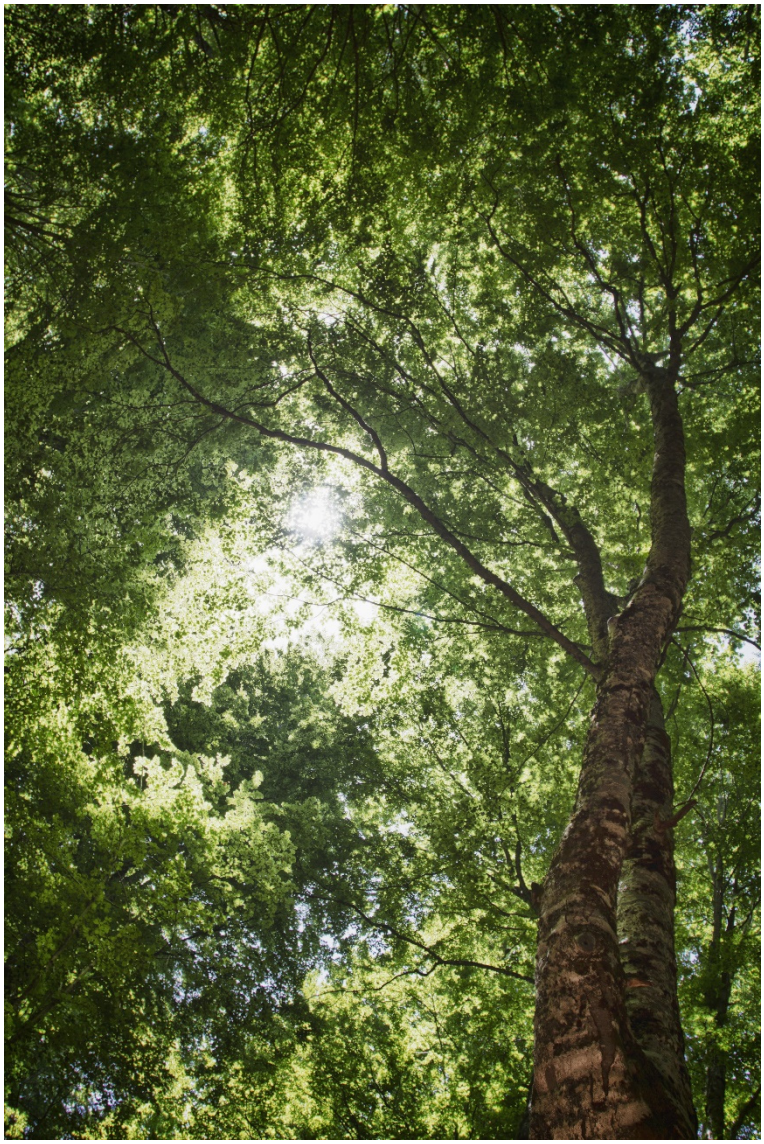
This study confirms the known strong relationship between nitrogen and residual weight (Berg and McClaugherty, 2014; Constantinides and Fownes, 1994; Coûteaux et al., 1995; Fioretto et al., 2005a; Kazakou et al., 2009; Melillo et al., 1982; Rutigliano et al., 2009; Taylor et al., 1989). N is shown to have a reduction of its concentration at the beginning of the decomposition process, and that happens because microbial N limitation can occur due to high microbial N demand (Moorhead and Sinsabaugh, 2006). The later increase of N during decomposition is due to the translocation of this element mainly by fungi from the soil and/or from the more decomposed layers of litter and the highest increase happens for all the litter that had a lower initial content of this nutrient (Cortez et al., 1996). It has to be highlighted that the content of N is shown to be higher in the leaves and soil of Pradaccio than Laceno, but the nitrogen mineralization has an inverse trend, being much higher in Laceno than Pradaccio. The higher nitrogen mineralization in Laceno could also be associated with the diverse pH, which implies a different bacteria:fungi ratio, being the latter more abundant on acidic soils (Bååth and Anderson, 2003; Cortez et al., 1996) or to the broader C:N ratio (Satti et al., 2003). Nitrogen mineralization has been showed to increase in microcosm experiments for the sole litter where, contrarily to the presented results, nitrification was much less (Cortez et al., 1996). On the

contrary, Brandstätter et al. (2013) report a similar pattern for nitrogen mineralization and show that sites with lower ammonium mineralization at the beginning have a lower mass loss, but N richer plants do not show higher decomposition rate. Admittedly, the higher contents of N in Pradaccio could be related to higher atmospheric deposition of nitrogen (Hyvönen et al., 2007) which have already been shown to be higher for beech woods of the northern Apennine than the ones in the south (Mosello et al., 2002). Leaves of Pradaccio tend to decompose faster in either soil likely according to their N because it poses a smaller difference in the C:N ratio between litter and decomposer microbes, thus allowing microbes to immobilize less N to decompose the plant material (Berg and McClaugherty, 2014).

In addition, our study confirms the importance of Mn in the decomposition process (Berg et al., 2006) which, according to our evidence, has a major role not only in the later stages of decomposition but also in the early ones. Undoubtedly, the discussed study suggests and endorses the importance of this nutrient for lignin-degrading fungi producing Mn-peroxidases (Berg and McClaugherty, 2014; de la Rubia et al., 2002; Kamitsuji et al., 2004; Osono, 2007; Palma et al., 2000). Fungi typically associated with Mn for the degradation of lignin are generally known to be Basidiomycota, which become predominant in fungal succession in the later stages, while the early stages are more dominated by Ascomycota, known cellulose or sugar fungi (Schneider et al., 2012). The dynamics of Mn availability is unknown, but may be related to its initial concentration (Berg et al., 2013, 2010), and this study confirms this trend which has been observed few times (Fioretto et al., 2001; Vesterdal, 1999). In fact, an increase of Mn is shown more noticeable in those leaves (Laceno) which had a lower Mn initial concentration. This suggests that, even from the early stage of decomposition, fungi colonizing the leaves recruit Mn from the environment (soil or more decomposed material) with a higher rate when this nutrient is limited in the litter. As proved by Joergensen et al. (2009), Mn strongly increased its concentration from the L1 to the F1 layers of a decomposition continuum (from 1.68 to 2.02 mg/g) but its concentration dropped in the F2 and H layers (0.45 to 0.21 mg/g). This evidence strengthens our suggestion that Mn is highly transported in the plant material when lignin starts its decomposition, while it becomes a source once lignin decay slows down because of the limiting effect of N in the higher decomposed layers. Additionally, in a climatic gradient, a highly significant and negative relationship between Mn concentration in the newly shed pine needle litter and mean annual temperature was found, suggesting that pine litter Mn concentration could be a consequence of local climate and that Mn appears to be the dominant factor determining limit values in pine ecosystems. A model connecting mean annual temperatures with Mn concentration for pine litter confirmed that higher local temperatures are associated with lower Mn concentrations (Berg et al., 2013, 2010, 1995). This could be true also for beech, and the differences in mean annual temperatures between Laceno and Pradaccio could be an explanation of the lower concentration of Mn for the hotter location and its lower decomposability under controlled condition. The lower concentration of Mn from this study compared to other studies could also contribute to explain the

differences between decomposition in mountain ecosystems like the Italian Apennines and those in Middle Europe, which generally prove to be faster. Although Berg et al. (2013) did not find a direct modelling effect of mean annual precipitation on Mn concentration in leaves, their study had few data regarding beech and none of them from mountain ecosystems. A correlation with the higher precipitation of mountain ecosystems and the lower concentration of Mn could be, instead, hypothesized.

4 Results from the seasonal analyses in Laceno and Pradaccio



Detail of a beech tree growing in Novacco, in the Pollino National Park (Calabria, Italy)

Table 4-1: Three-way ANOVA for the quantity and quality parameters of the seasonal sampling.

	Organic Matter			Nitrogen			C:N			Cellulose			Lignin		
	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.
Location	39.9	**<0.001**	3.4	35.7	**<0.001**	4.0	32.7	**<0.001**	4.6	6.9	**0.009**	8.0	5.9	**0.016**	11.7
Season	3.4	**0.036**	0.3	290.0	**<0.001**	32.2	770.3	**<0.001**	66.9	12.7	**<0.001**	14.7	0.9	0.428	1.7
Layer	1104.7	**<0.001**	93.1	528.4	**<0.001**	58.7	274.2	**<0.001**	23.8	52.2	**<0.001**	60.4	34.0	**<0.001**	67.1
Location × Season	0.2	0.801	0.0	6.0	**0.003**	0.7	8.0	**<0.001**	0.7	7.1	**0.001**	8.2	1.6	0.215	3.1
Location × Layer	36.3	**<0.001**	3.1	35.5	**<0.001**	3.9	33.5	**<0.001**	2.9	3.0	**0.009**	3.4	3.4	**0.004**	6.6
Season × Layer	1.2	0.310	0.1	2.0	**0.025**	0.2	5.8	**<0.001**	0.5	4.2	**<0.001**	4.8	3.4	**<0.001**	6.7
Loc. × Seas. × Lay.	1.0	0.467	0.1	2.1	**0.022**	0.2	7.0	**<0.001**	0.6	0.3	0.979	0.4	1.6	0.095	3.1

4.1 Quality and quantity of organic matter

Unsurprisingly, the concentration of organic matter (O.M.) (Fig. 4-1) along the decomposition continuum of the plant litter was always much higher than in the soil. In the litter, a decrease between the Li layer and the Lhf layer c is observable. Although the content of O.M. appears to be higher in Pradaccio than Laceno, a sharper decrease in the Lhf of Laceno is seen, and this is clearly in concordance with the faster decomposition trend already shown for this location. In the soil, Pradaccio shows a very high content of O.M. only on the first 5 cm, while deeper the concentration is stable around 10%. In Laceno, instead, the concentration of O.M. is less in the upper layer, but higher deeper in the soil, where even at a depth 30-40 cm a concentration of circa 150 mg/g can be seen. Three-Way ANOVA (Tab. 4-1 for all the ANOVAs in section 4.1) shows that the factor more influencing the variability of O.M. is Layer, which is obvious given the large differences between litter and soil. Yet, also the factor Location explains a significant 3.4% of the total variance, while the factor Season is significant, but it explains only 0.3% of the variance. Holm-Sidak post-hoc test (data not shown) points out that the seasons differing significantly are autumn vs. summer (p=0.030). It is worth to notice, for instance, that in the soil of Laceno, in the deeper layers (15-30 to 30-40 cm), there is a slight increase in the O.M. between autumn and summer while in Pradaccio, in the 0-5 cm, there is a sudden increase in the O.M. between autumn and spring. This last phenomenon can be followed and observed also in other qualitative/quantitative parameters of the O.M. and in the enzymes activities. It is not clear if this increase in the O.M. is due to natural causes, anthropic disturbance (forestry management) or a combination of both.

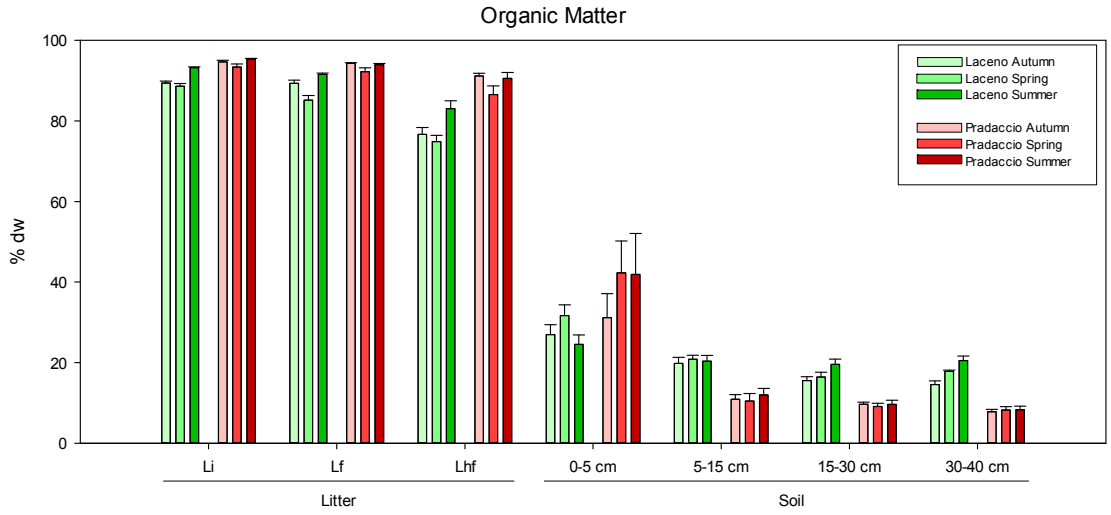


Figure 4-1: Concentration of organic matter in the seasonal sampling. Values are means±s.e. (n=6).

Among the nutrients, nitrogen and carbon have been measured for both litter and soil, while the other elements have been analysed only for the litter and will be discussed separately later in the chapter. As for nitrogen (Fig. 4-2), two things are clearly evident, which both concurs to the on-the-field decomposition experiment. First, the concentration of N in the litter of Pradaccio was higher than Laceno and, second, there was a clear trend for increase along the decomposition continuum and along the seasons. In the soil, the litter's trend was reflected only in the upper 0-5 cm layer, while deeper in the soil the trend of nitrogen reflected more clearly the one from the O.M.. The Three-way ANOVA responds to the remarks above. The factor explaining the higher amount of variance (59%) is Layer, but also Season is highly significant and explains a large 32% of total variance. Factor Location is significant, but it explains a lesser degree of variance. All the interactions between the factors are also significant, which highlights the complexity of the trend of N between locations, seasons and layers. In general, the most important information is the outstanding increase in N during the seasons, which is in magnitude much higher than what happened in the litterbags.

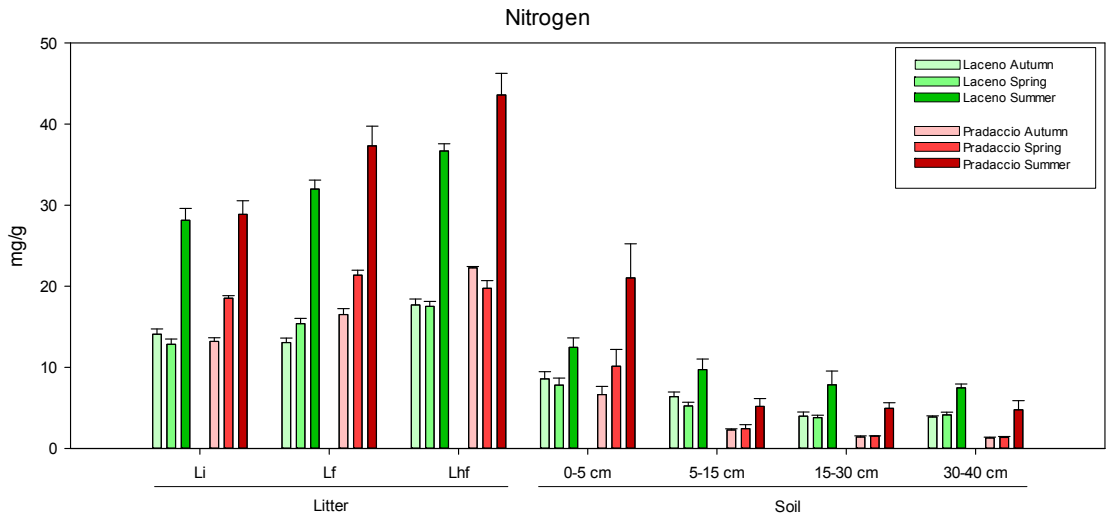


Figure 4-2: Concentration of nitrogen in the seasonal sampling. Values are means±s.e. (n=6).

The carbon to nitrogen ratio shows a trend which is, predictably, opposite to the trend of N (Fig. 4-3). The ratios are different between the seasons more than between the locations, although it can be noted that, in the autumn Li layer of the litter, the ratio is higher in Pradaccio than Laceno. Later on, it can be always distinguished the drastic reduction of the C:N ratio when we get to the summer samples. Differently from N, the ANOVA for C:N points out that the factor explaining most of the variance is Season (67%) while Layer explains only 24% of the total variance. All the factors from the ANOVA are significant, implying a rather complex and unambiguous interpretation of the trends even though, as stated, seasons are the variables explaining most of the variability.

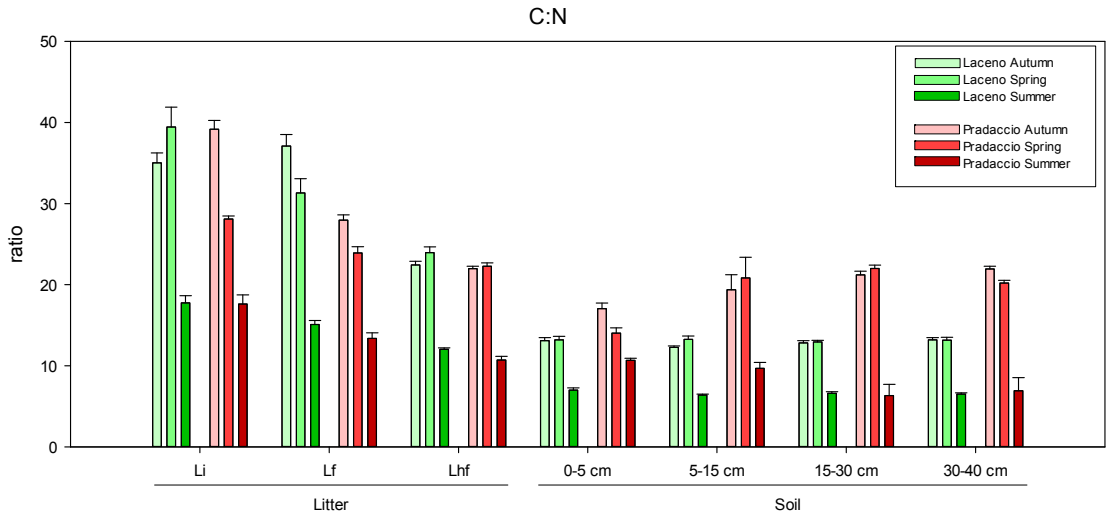


Figure 4-3 C:N ratio in the seasonal sampling. Values are means±s.e. (n=6).

As a qualitative feature of organic matter, content of cellulose and lignin are presented as percentage of total O.M. (Fig. 4-4). Thus, it is implied that the absolute concentration of these compounds on a dry matter basis is much higher in the leaf litter than in the soil. Cellulose represents in the litter almost 60% of the organic matter in the litter during autumn in the Li layer and it reaches 40% of the O.M. in the Lhf layer during summer. In the soil, its concentration drops to usually less than 5% of the O.M., except for the 0-5 cm layer. Lignin, on the contrary, increases almost linearly along the decomposition continuum and soil profile, becoming by far the most abundant component of O.M. in the deeper layers of the soil, especially for Pradaccio. At least in the litter, the contents of cellulose and lignin are directly connected to each other through a negative correlation (Fig. 4-5). A clear seasonal pattern can be seen also for these variables, with a decrease in cellulose and an increase in lignin from autumn to summer. Accordingly, Season is the factor explaining most of the variance for both variables (93% for cellulose and 67% for lignin), even though, especially for lignin, there is a clear significant difference also for the factor Location. It can be noted that in the deeper layers of Pradaccio (from 5-15 cm onwards) lignin is more abundant and, in contrast to all other trend, it was more present in autumn than summer.

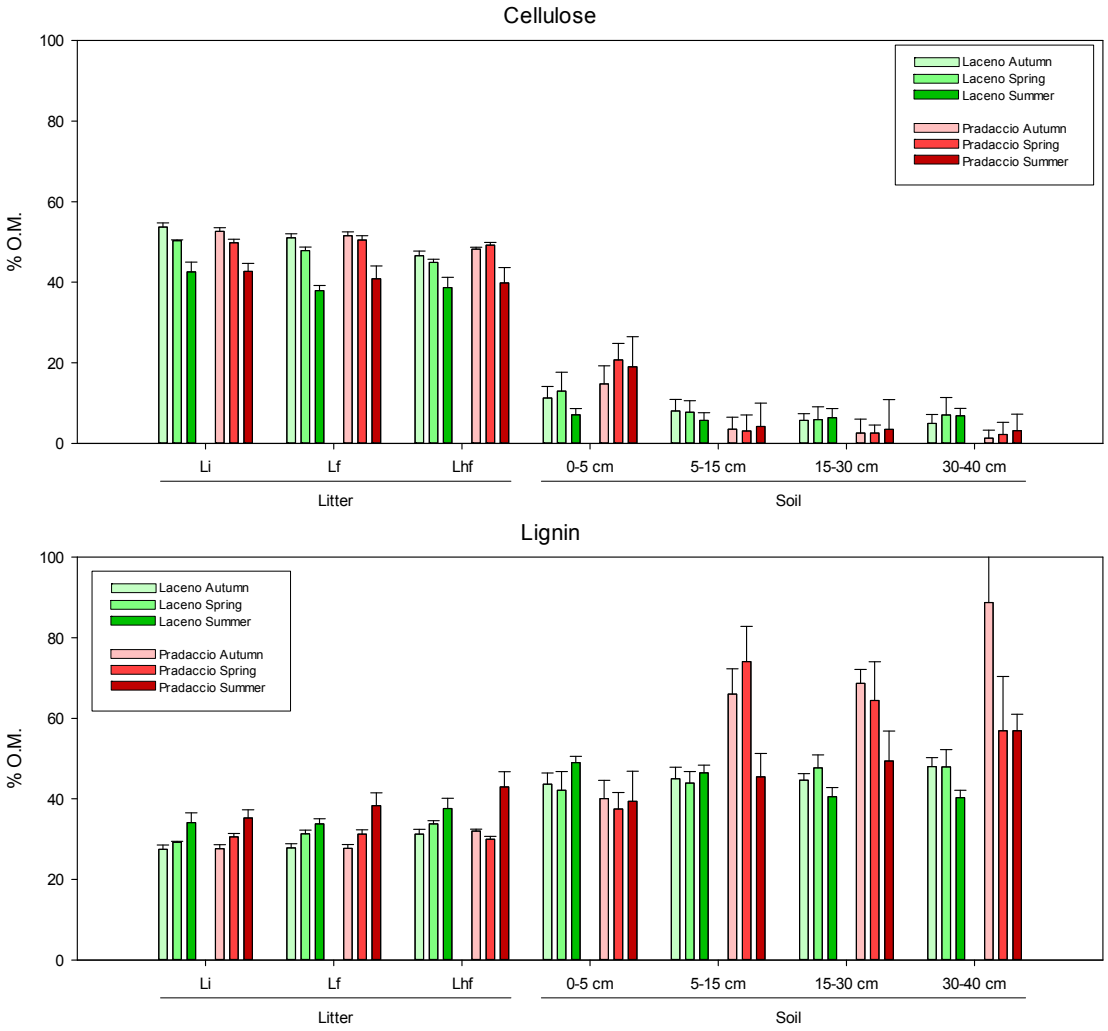


Figure 4-4: Concentration of cellulose (above) and lignin (below) expressed as function of the O.M. in the seasonal sampling. Values are means±s.e. (n=6).

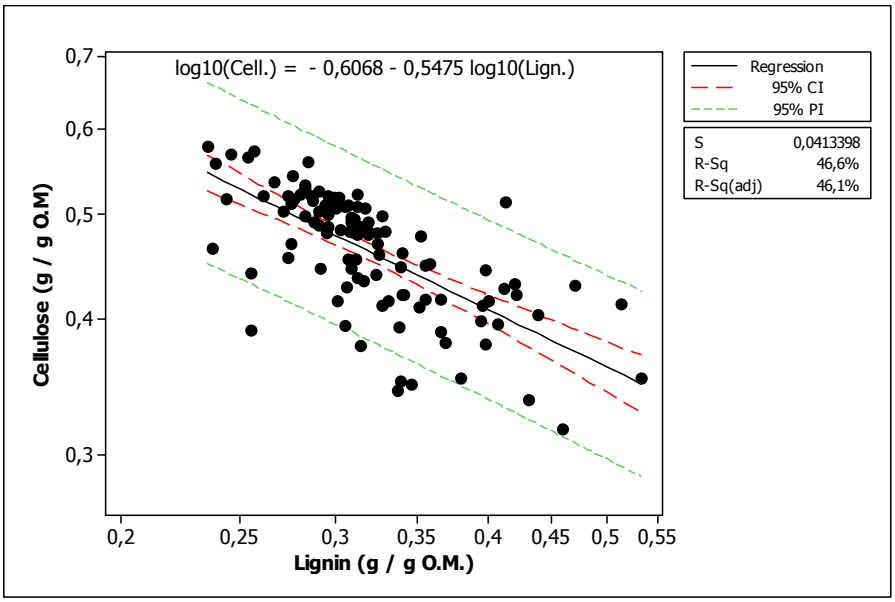


Figure 4-5: Linear regression using Cellulose (on log10 transformed data) as dependent variable and Lignin (on log10 transformed data) as predictor for the litter. Red lines represent 95% confidence interval, while green lines represent 95% prediction interval.

4.2 Nutrients along the leaf litter decomposition continuum

Table 4-2: Three-way ANOVA for the nutrients in the leaf litter decomposition continuum in the seasonal sampling.

	P			K			Ca			Mg			Fe			Mn		
	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.
Location	193.7	**<0.001**	25.0	2.2	0.139	5.5	452.9	**<0.001**	94.8	33.7	**<0.001**	24.6	0.5	0.482	0.9	77.4	**<0.001**	34.1
Season	510.2	**<0.001**	65.8	28.3	**<0.001**	69.3	11.9	**<0.001**	2.5	46.4	**<0.001**	33.9	32.2	**<0.001**	58.8	117.1	**<0.001**	51.6
Layer	52.2	**<0.001**	6.7	5.5	**0.006**	13.4	2.7	0.070	0.6	47.8	**<0.001**	34.9	11.7	**<0.001**	21.3	27.6	**<0.001**	12.1
Location × Season	10.1	**<0.001**	1.3	1.0	0.389	2.3	2.8	0.063	0.6	0.7	0.484	0.5	0.3	0.740	0.6	2.3	0.109	1.0
Location × Layer	0.7	0.487	0.1	0.8	0.440	2.0	4.8	**0.010**	1.0	1.2	0.317	0.9	0.3	0.745	0.5	0.8	0.467	0.3
Season × Layer	4.8	**0.001**	0.6	2.1	0.088	5.1	1.0	0.394	0.2	6.4	**<0.001**	4.6	9.6	**<0.001**	17.5	1.7	0.155	0.8
Loc. × Seas. × Lay.	3.1	**0.019**	0.4	0.9	0.442	2.3	1.4	0.233	0.3	0.8	0.531	0.6	0.3	0.907	0.5	0.2	0.910	0.1

With the exception of S, all the nutrients which have been analysed in the litterbags have been measured also along the leaf litter decomposition continuum, although here the concentrations will be expressed only as mg/g of dry litter.

As for P (Fig. 4-6), it can be observed that it tends to increase along the decomposition continuum and has a peak during summer for both locations. Similarly to the newly shed leaves (see Tab. 3.6 for all references to newly shed leaves), the concentration is generally higher in Laceno than Pradaccio. The values reached in the summer Lfh layer appear to be lower than what happened in on-the-field litterbags. Three-way ANOVA (Tab. 4-2 for all the ANOVAs in section 4.2) confirms that the factor explaining most of the variance is Season (66%) while Location explains 25% of the variance.

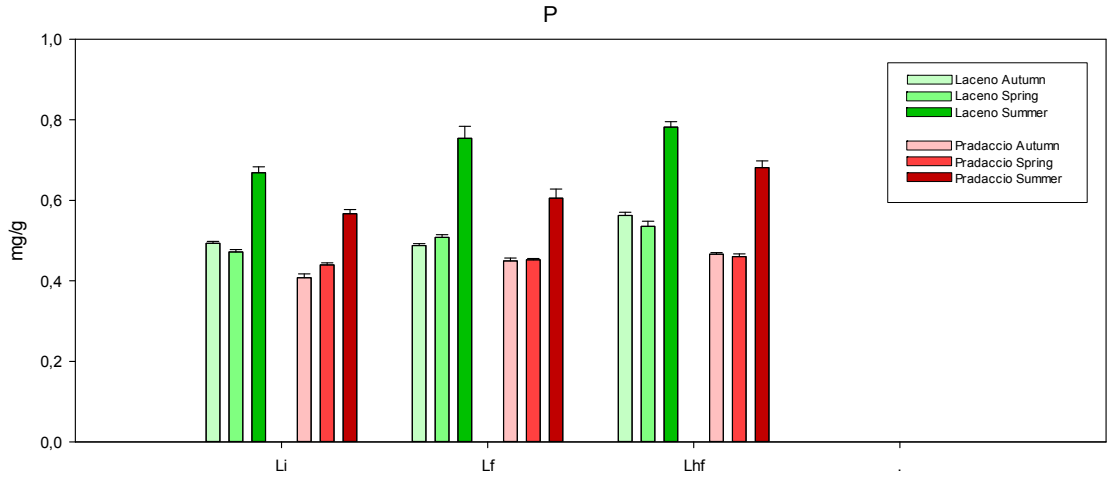


Figure 4-6: Concentration of P in the leaf litter decomposition continuum of the seasonal sampling. Values are mean±s.e. (n=6).

The initial concentration of K in the two newly shed litter was similar, although the difference existing was significant. Nevertheless, in the field no particular difference in K (Fig. 4-7) can be detected between the locations, and this is confirmed by the ANOVA. Only for summer there is a

peak of K, and the factor Season explains 69% of total variance. Anyway, the concentration of K is clearly less than the newly shed litter, confirming the leaching and high mobility of this nutrient. The trend appears to be generally overlying the one in the litterbags.

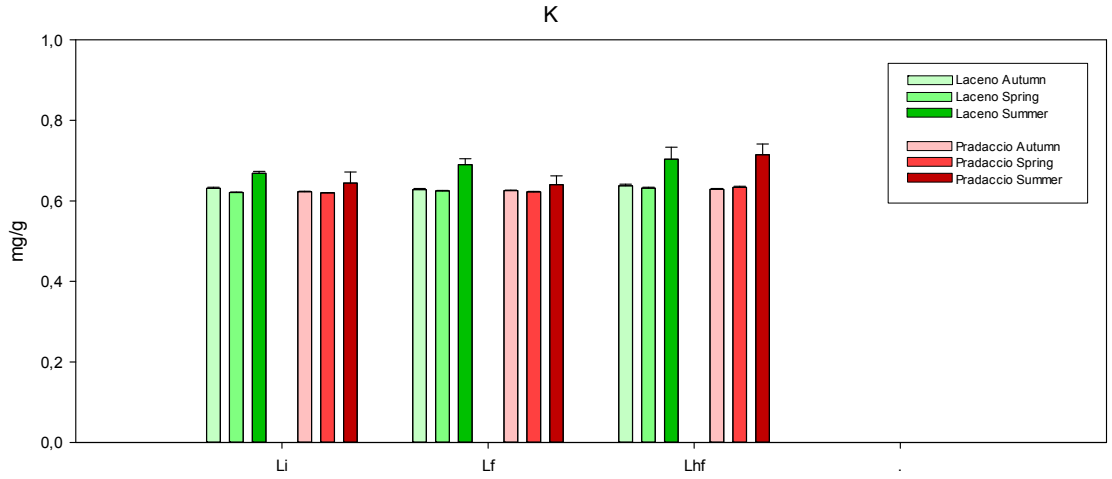


Figure 4-7: Concentration of K in the leaf litter decomposition continuum of the seasonal sampling. Values are mean±s.e. (n=6).

Ca (Fig. 4-8) initial concentration in the newly shed litter was different between Laceno and Pradaccio (16 vs. 13 mg/g, respectively) and this difference can be observed also along the decomposition continuum (95% of total variance in the ANOVA is explained by factor Location). As a matter of fact, Laceno's litter has always a higher concentration of Ca than Pradaccio the concentration remain relatively stable between and within seasons and layers. An exception is Laceno, where a slight trend to increase of Ca during the seasons can be seen (the Location × Layer factor in the ANOVA explains 1% of total variance, but it is significant).

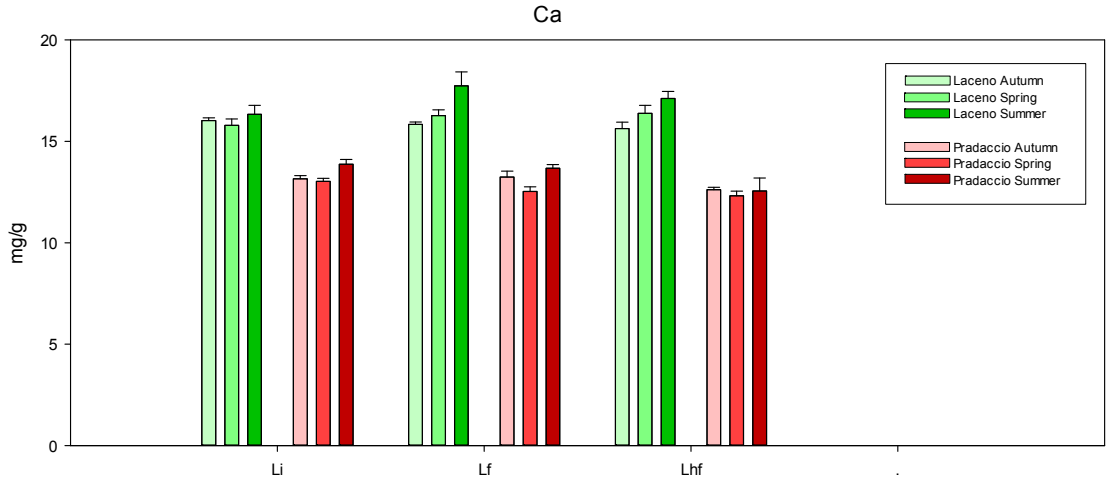


Figure 4-8: Concentration of Ca in the leaf litter decomposition continuum of the seasonal sampling. Values are mean±s.e. (n=6).

A very similar trend to Ca is expressed by Mg (Fig. 4-9). This is confirmed also in the litterbags experiment, where both elements, due to the similar leaching phenomena, appeared to have comparable trends. In almost all cases, there is a low decrease in Mg between autumn and spring and

then an increase during summer. Although, as said, the trend was similar to Ca, the Three-way ANOVA points out an almost even distribution of the variance between the factors Location, Season and Layer.

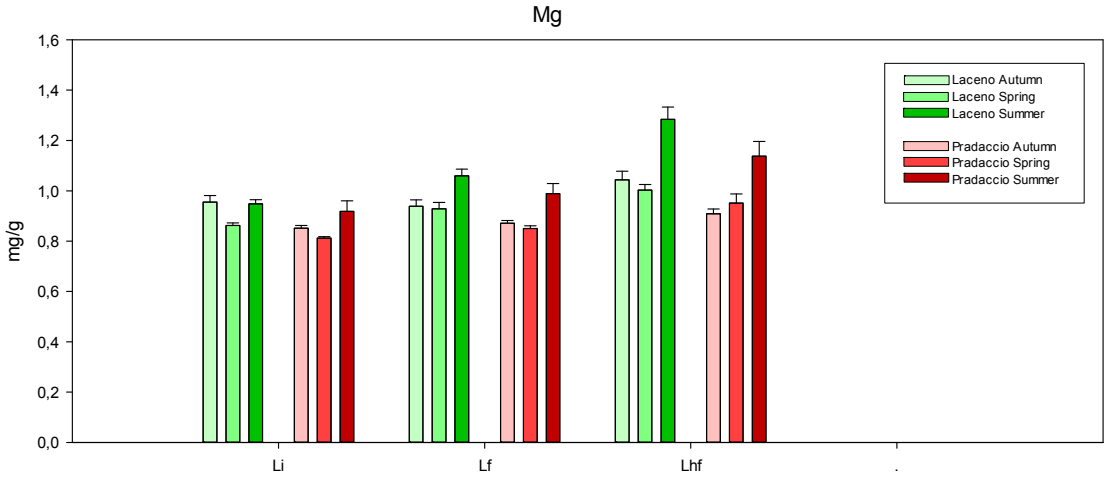


Figure 4-9: Concentration of Mg in the leaf litter decomposition continuum of the seasonal sampling. Values are mean±s.e. (n=6).

As for Fe (Fig. 4-10), the two newly shed litters did not have significant differences but, as was already noted in the litterbags, the concentration of this element increases substantially during time. Especially in the Lhf layer of both locations, there is a remarkable increase of Fe. The trend, neat of certain numerical differences, is completely comparable to what has been observed for the litterbags. The ANOVA confirms these results, with factor Season and Layer explaining 58% and 21% of the variance, respectively. No significant difference is noted between the locations, but a highly significant interaction between Season and Layer can be appreciated, explaining 17% of the variance.

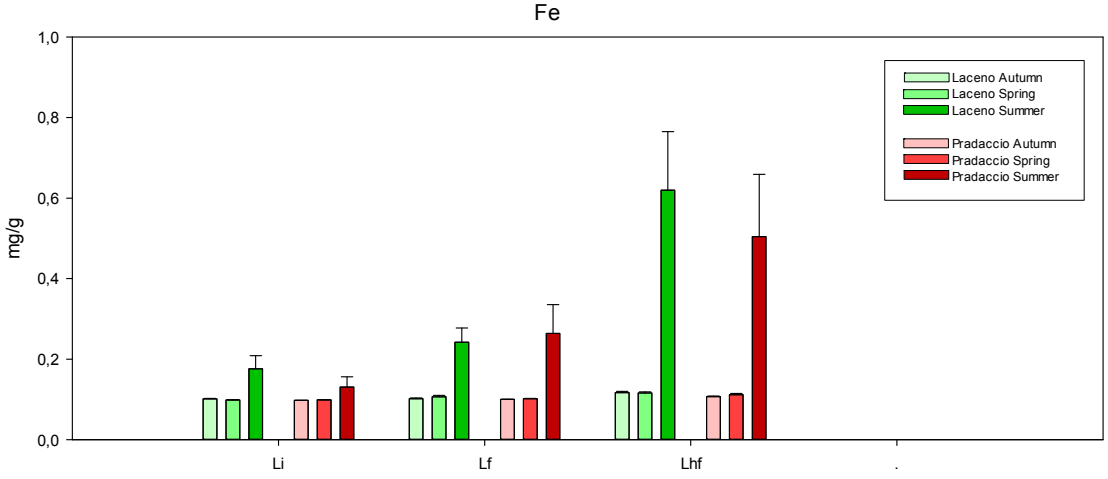


Figure 4-10: Concentration of Fe in the leaf litter decomposition continuum of the seasonal sampling. Values are mean±s.e. (n=6).

In the litterbags experiment the substantial importance of Mn in the decomposition process was pointed out. Accordingly, the concentration of Mn (Fig. 4-11) increases from Li to Lhf for both sites (keeping a substantial difference between Pradaccio and Laceno in relation to the higher initial

content of the element for the first site) and has a peak during summer. The ANOVA underlines that the factor explaining most of the variance is Season (51%), while Location explains 34% of the variance and layer 12% of the variance. In accordance to what happened in the litterbags, considering that the initial concentration of Mn in the newly shed material was 63 µg/g in Laceno and 140 µg/g in Pradaccio, the immobilization of Mn in Laceno is substantially greater, with Mn reaching 2 times the initial concentration in Lhf during summer, while for Pradaccio the concentration is just slightly above the initial values.

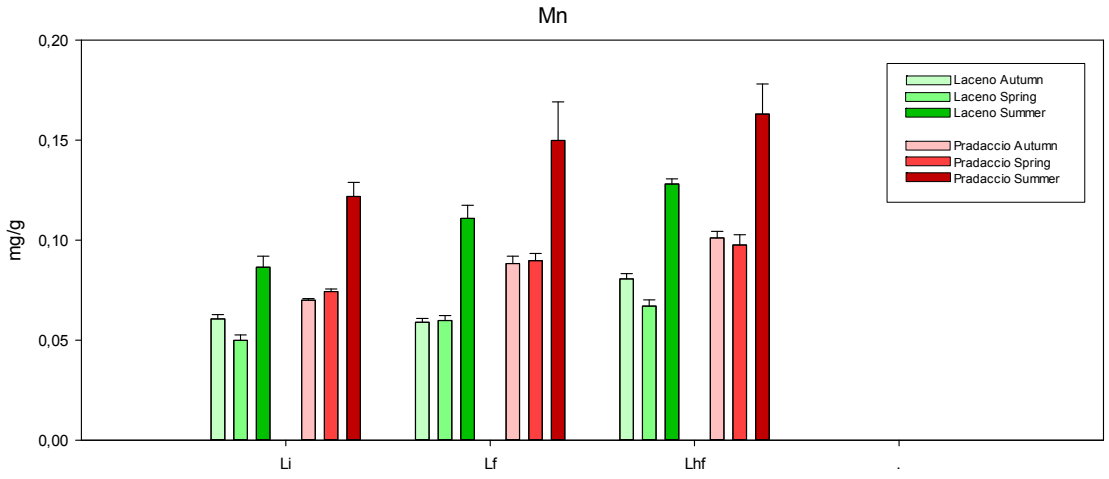


Figure 4-11: Concentration of Mn in the leaf litter decomposition continuum of the seasonal sampling. Values are mean±s.e. (n=6).

4.3 Enzyme dynamics

Table 4-3: Three-way ANOVA for the enzyme dynamics of the seasonal sampling.

	Cellulase			Xylanase			Laccase			Peroxidase			Acid Phosph.			Alkaline Phosph.			Chitinase			Dehydrogenase		
	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.	F	*p*	% Var.
Location	1.8	0.178	0.1	7.6	**0.006**	0.5	89.9	**<0.001**	63.2	71.8	**<0.001**	39.9	31.6	**<0.001**	25.4	0.1	0.782	0	116.3	**<0.001**	28.9	3.4	0.068	2.2
Season	56	**<0.001**	4	84.1	**<0.001**	5	9.6	**<0.001**	6.8	5.1	**0.007**	2.8	2.9	0.058	2.3	108.4	**<0.001**	57.8	141.8	**<0.001**	35.2	19	**<0.001**	12.3
Layer	1264.3	**<0.001**	90.3	1492.5	**<0.001**	89.5	12.3	**<0.001**	8.6	52.2	**<0.001**	29	18.8	**<0.001**	15.1	64.2	**<0.001**	34.2	124.5	**<0.001**	30.9	87.8	**<0.001**	56.8
Location × Season	20.5	**<0.001**	1.5	8	**<0.001**	0.5	19.6	**<0.001**	13.8	22.5	**<0.001**	12.5	36	**<0.001**	28.9	1.6	0.215	0.8	8.3	**<0.001**	2.1	27	**<0.001**	17.5
Location × Layer	13.9	**<0.001**	1	20.4	**<0.001**	1.2	3.1	**0.006**	2.2	20.9	**<0.001**	11.6	12.9	**<0.001**	10.3	1.7	0.135	0.9	7.3	**<0.001**	1.8	4.7	**<0.001**	3
Season × Layer	39.6	**<0.001**	2.8	52.2	**<0.001**	3.1	6.3	**<0.001**	4.5	4.4	**<0.001**	2.4	15.5	**<0.001**	12.4	5.8	**<0.001**	3.1	2.2	**0.013**	0.5	7.8	**<0.001**	5
Loc. × Seas. × Lay.	3.5	**<0.001**	0.3	2.2	**0.011**	0.1	1.3	0.205	0.9	3	**<0.001**	1.7	6.7	**<0.001**	5.4	5.8	**<0.001**	3.1	2.3	**0.008**	0.6	4.9	**<0.001**	3.2

As already noticed in the litterbags experiment, there is a close relationship between the enzymes decomposing cellulose (cellulase and xylanase) and those decomposing lignin (laccase and peroxidase) with variations related to the decrease and increase of these two components, respectively. As for cellulase and xylanase (Fig. 4-12), there is an outstanding fall of the activity between litter and soil. Besides, there is a clear seasonal pattern, with the activities decreasing both from autumn to summer and along the decomposition continuum. The activities are comparable between the sites, and the factor explaining most of the variance is Layer, justifying 90% of the variance for both cellulase and xylanase (Tab. 4-3 for all the ANOVAs in section 4.3).

Laccase and peroxidase follow a different trend (Fig. 4-13). There is not such a great difference of activity between litter and soil and, for peroxidase in Pradaccio, the activity is even higher in the soil than in the litter. The explanation is clearly the presence of almost the sole lignin in the deeper layers of the soil. There is a general difference in the seasonal pattern between the locations, with Laceno having usually higher activities during autumn and Pradaccio higher activities during summer. This may reflect the differential microbial response to climate between the two locations, which has been observed in both the field and laboratory. Similarly, a temporal-spatial diversity of laccase and peroxidase can be noted, with laccase more abundant in the Lf layer and peroxidase in the Lhf.

The relationship between cellulose and cellulose-degrading enzymes (xylanase is reported in the figure) is more evident in the litter (R^2adj.=0.534), whereas for lignin and lignin-degrading enzymes (peroxidase in figure) a closer correlation can be seen in the soil (R^2adj.=0.191) (Fig. 4-14).

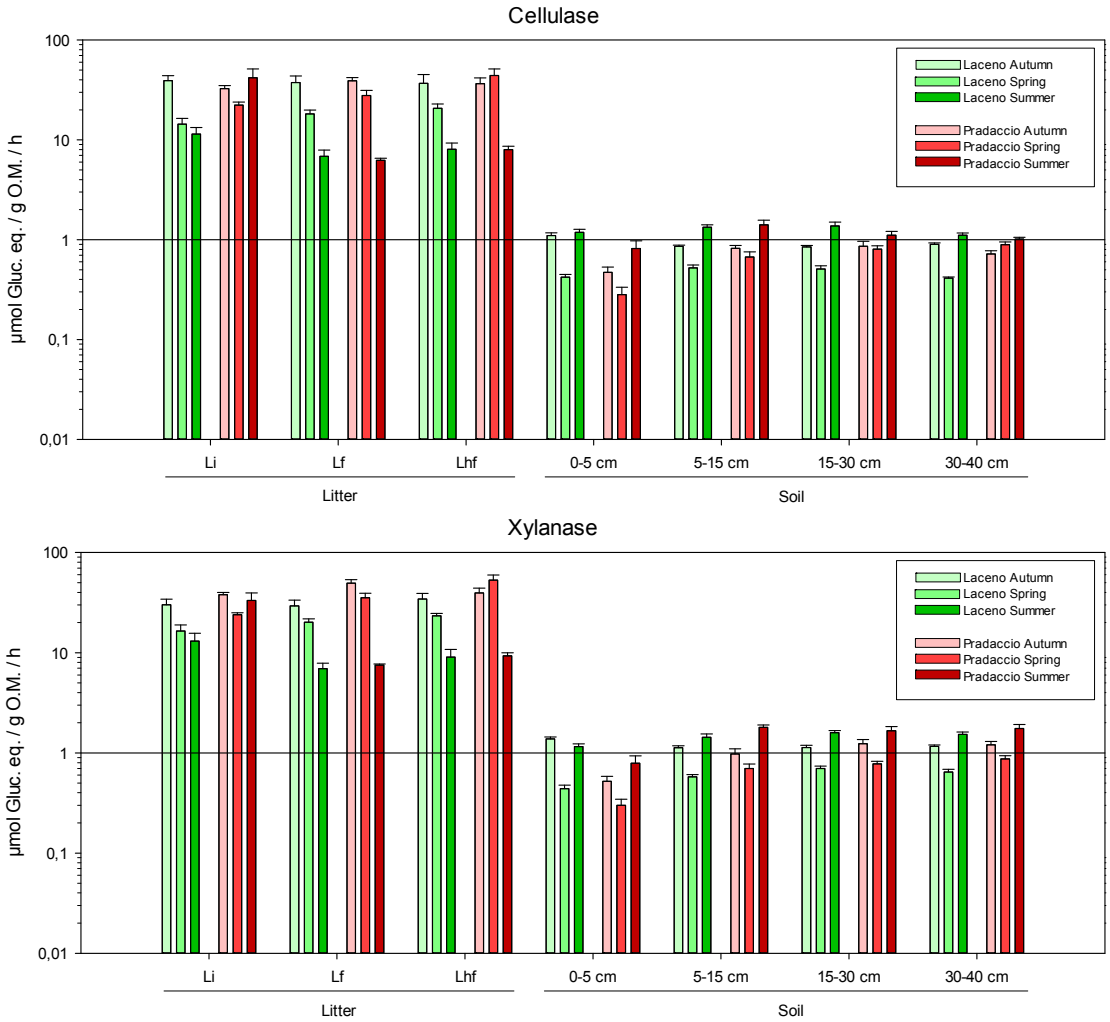


Figure 4-12: Activity of Cellulase (above) and Xylanase (below) in the seasonal sampling. Values are mean±s.e. (n=6).

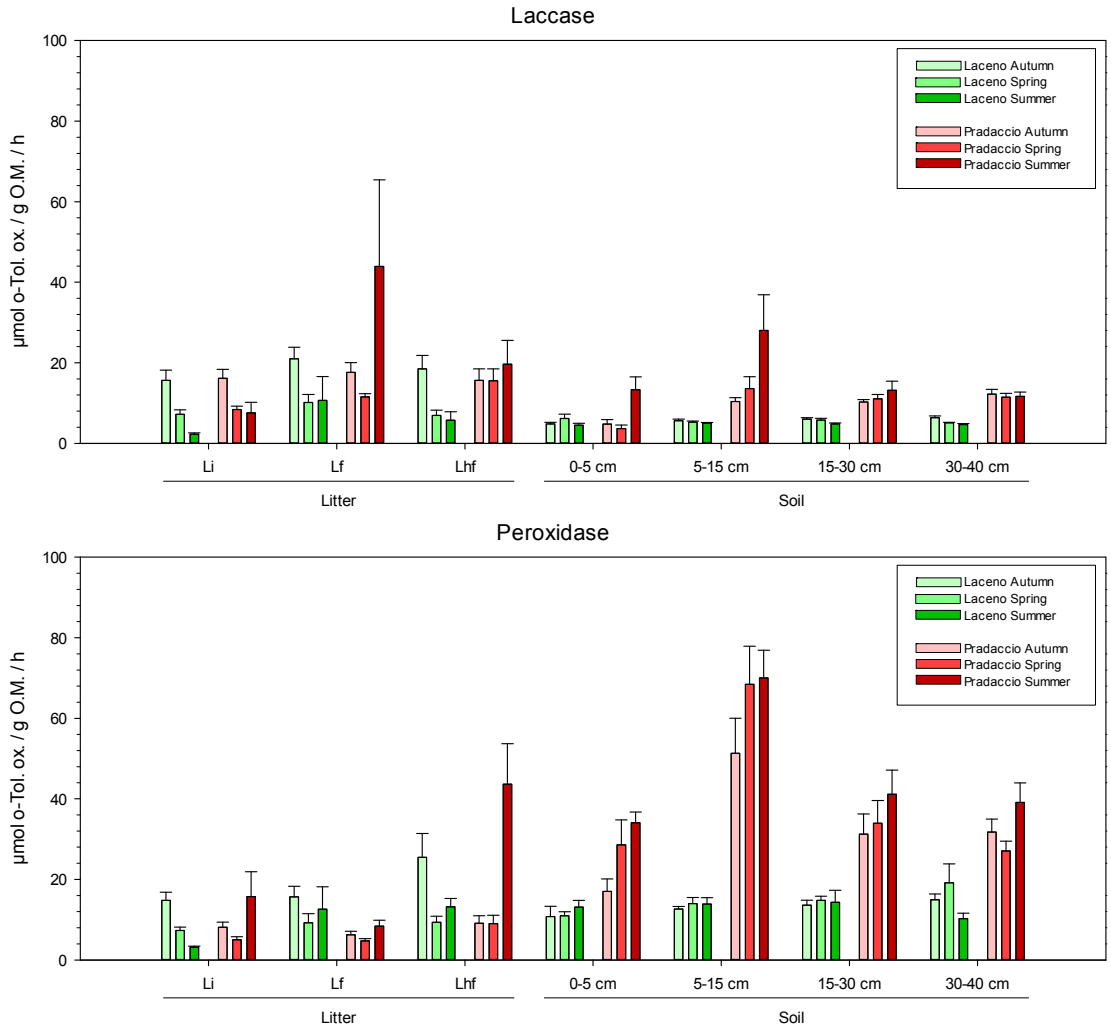


Figure 4-13: Activity of Laccase (above) and Peroxidase (below) in the seasonal sampling. Values are mean±s.e. (n=6).

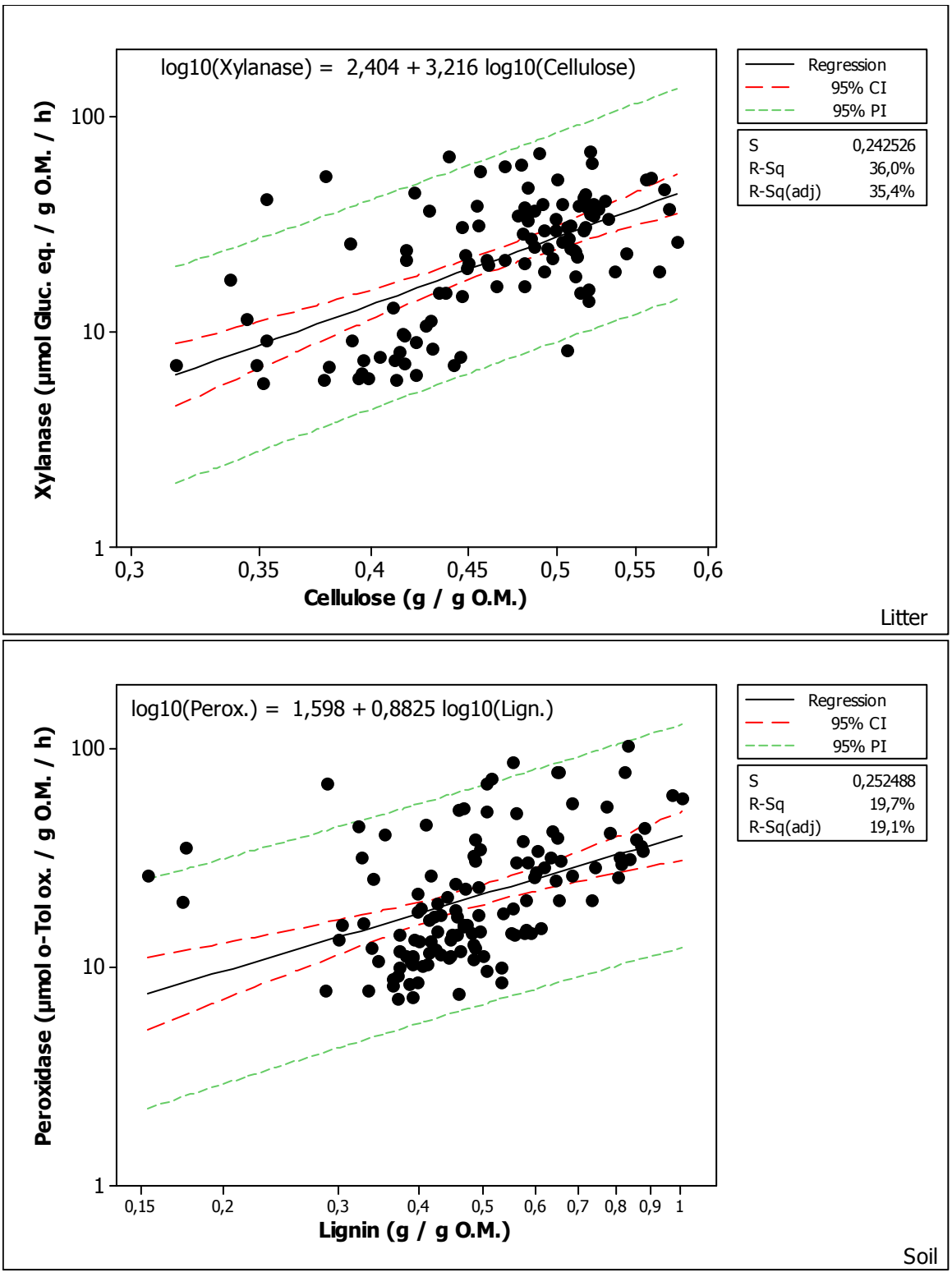


Figure 4-14: Linear regression for the litter (above) using Xylanase (on log10 transformed data) as dependent variable and Cellulose (on log10 transformed data) as predictor. Linear regression for the soil (below) using Peroxidase (on log10 transformed data) as dependent variable and Lignin (on log10 transformed data) as predictor. Red lines represent 95% confidence interval, while green lines represent 95% prediction interval.

The trend of acid phosphomonoesterase appears to be complex (Fig. 4-15 above). In the litter, there is an increase of activity along the decomposition continuum, which is in general concordance with what happened in the litterbags. Nevertheless, in contrast to other chemical and biological

parameters of this experiments, the activity generally decreases from autumn to summer. On the contrary, in the soil, the activity follows a trend similar to peroxidase, with a maximum activity in the deeper layers of the soil (15 to 40 cm) and, once again, generally higher from autumn to summer. Clear differences can also be noticed between Laceno and Pradaccio, and the complexity of these differences are explained also in the ANOVA, where the factor explaining most of the variance (29%) is Location×Season. Alkaline phoshomonoesterase has a simpler trend (Fig. 4-15 below). The activity appears to be more or less constant along the decomposition continuum, with activity decreasing from autumn to summer. In the soil, the activity is much stronger the deeper the soil gets, there is a peak in autumn, a decrease in spring and a reprise of the activity during summer, although not to the level of autumn. Accordingly, the factor expressing most of the variance is Season (58%) followed by Layer (34%).

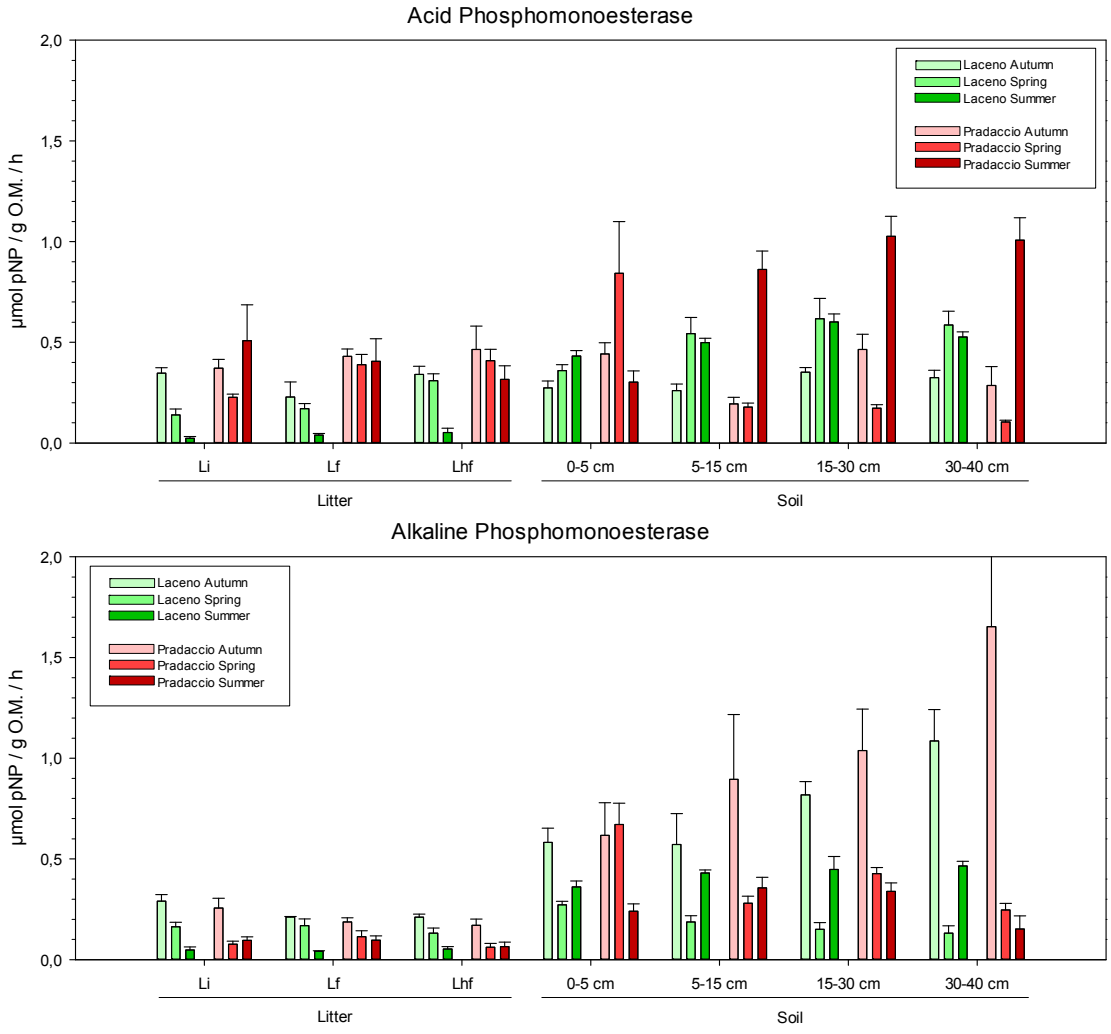


Figure 4-15: Activity of Acid (above) and Alkaline (below) Phosphomonoesterases in the seasonal sampling. Values are mean±s.e. (n=6).

Chitinase activity is, similarly to cellulase and xylanase, much more expressed in the litter than in the soil (Fig. 4-16). Like phosphomonoesterases, it is generally more expressed during autumn and it is more abundant in Lf and Lhf layers. In the soil, its presence is much reduced, although the

values are comparable (and sometimes higher) than the activity in the summer of Laceno. Also, there is a higher expression of this enzyme during autumn and the values of Pradaccio are generally higher than those of Laceno. The variance in the ANOVA is distributed between factors Season (35%), Layer (31%) and Location (29%). As for the litterbags, a good relationship between Chitinase and N is expressed considering the data of both litter and soil, with a low R^2adj. (0.144) but a high significance of the regression equation (p<0.001) (Fig. 4-17).

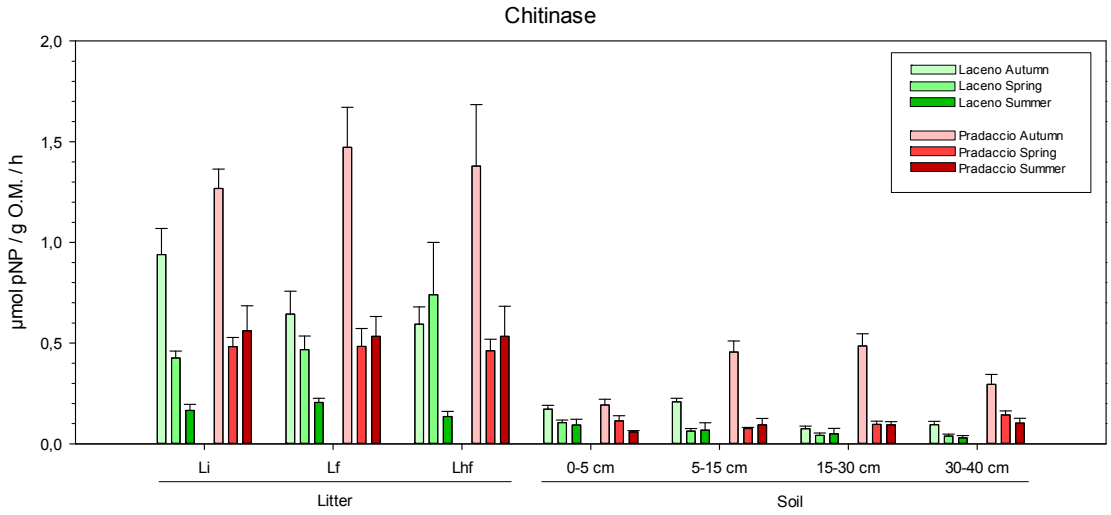


Figure 4-16: Activity of Chitinase in the seasonal sampling. Values are mean±s.e. (n=6).

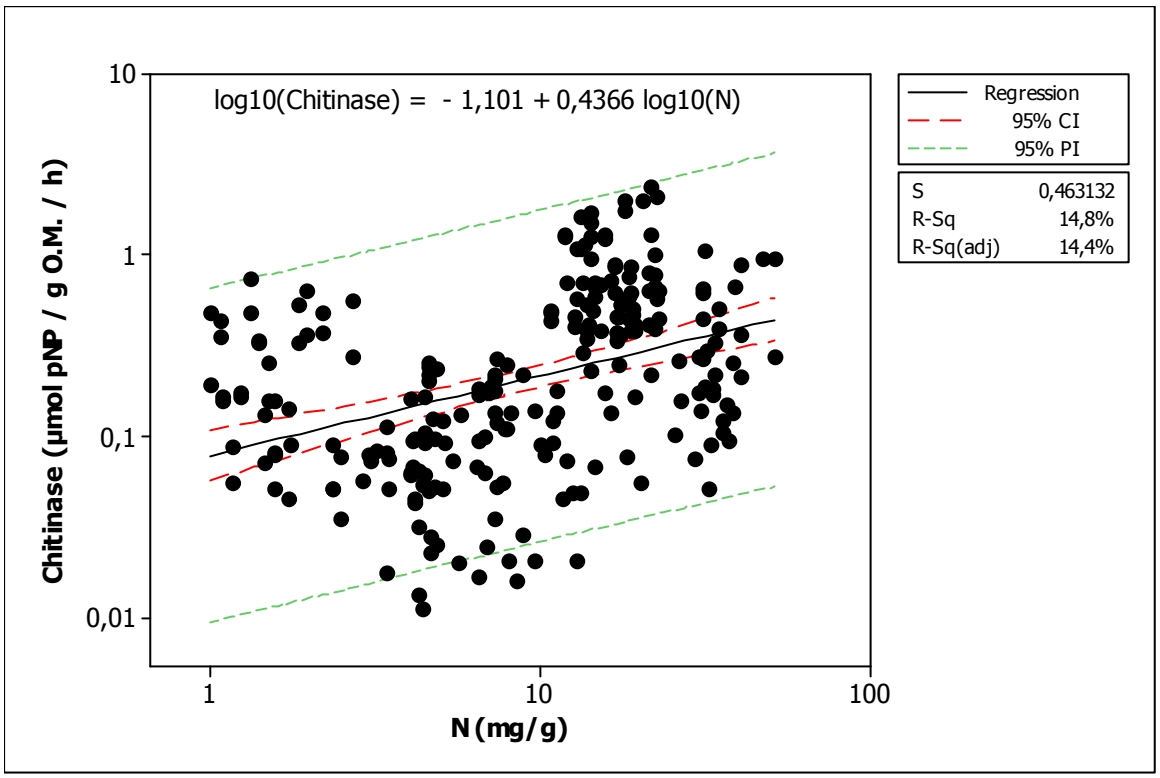


Figure 4-17: Linear regression for both litter and soil using Chitinase (on log10 transformed data) as dependent variable and N (on log10 transformed data) as predictor. Red lines represent 95% confidence interval, while green lines represent 95% prediction interval.

Dehydrogenase, as already mentioned, is a good descriptor of total microbial activity related to the presence of organic matter. Although the values are generally higher for the litter (and increasing along the decomposition continuum), the enzyme is well expressed also in the soil (Fig. 4-18). There is not a great difference of activity between Laceno and Pradaccio, but a different pattern is visible among Location×Season (ANOVA explains 18% of variance for this interaction). In Laceno, the microbial activity appears to be higher in Li layer during autumn, while in all other cases the enzyme is more expressed in autumn (for Pradaccio) or spring (for Laceno) and less abundant in summer. In Pradaccio, the enzyme is generally more active in autumn in the litter, while in the soil (with the exception of the 15-30 cm layer) is more present during spring or summer.

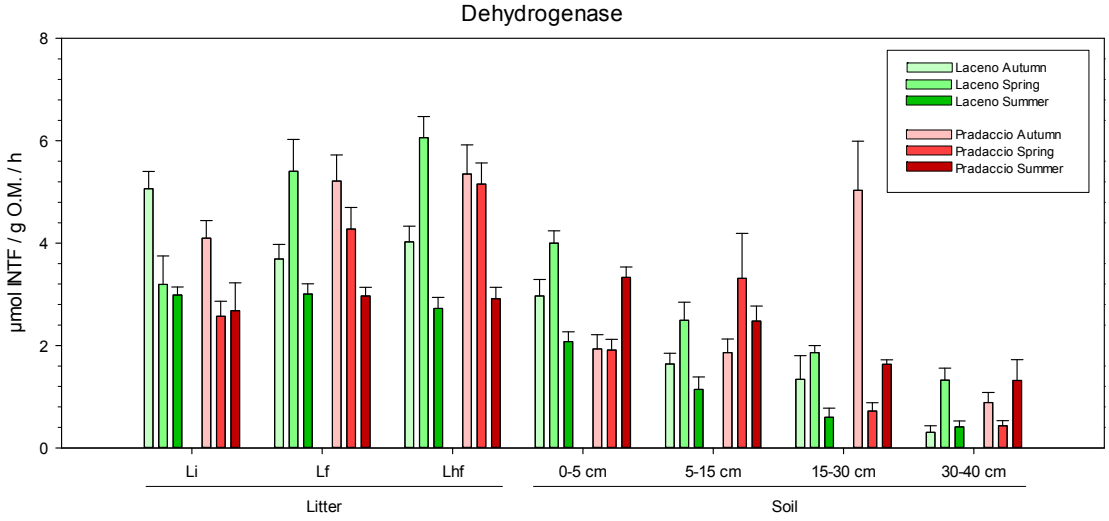


Figure 4-18: Activity of Dehydrogenase in the seasonal sampling. Values are mean±s.e. (n=6).

4.4 Multivariate analysis

In order to achieve a comprehensive interpretation of the dataset, a multivariate analysis of the data has been done using a principal component analysis (PCA) computed on the correlation matrix (i.e. after standardization of the data). Sub-datasets have been created per each of three layers of the litter and the soil's four, with points coloured in the scree plots according to location and season. Only the first two principal components are shown, which together usually explain more than 50% of the overall variance. Being summer the most discriminant season, the points from both locations in this season are highlighted and a red line aids to visually separate the two locations within this season.

In the litter (Fig. 4-19), there is clear evidence of diversity from the data of the summer season, no matter the decomposition layer. The loading plots show that the diversity of the summer sampling is due mostly to chemical quality of the plant material, namely nutrient content (with the exception of Ca) and lignin content. It is evident, throughout the decomposition continuum, that Pradaccio has

higher contents of mostly N and Mn while Laceno is different for the P, Mg and Fe contents. The autumn/spring points are more diversified from the microbial enzyme activities and the cellulose content. Moreover, whereas in the Li layer there is a certain vicinity of the autumn/spring points of Laceno and Pradaccio, during the decomposition continuum, especially in the Lf layer, the two locations form separate blocks in the scree plot. Accordingly, Laceno is distinguished by its C:N ratio and dehydrogenase, while Pradaccio is driven by acid phosphomonoesterase and chitinase. Finally, we can see that the first principal component explains a greater amount of variance the more we proceed on the decomposition continuum (from 36% to 47%) and this implies that, while in the Li layer there are not so many variables overriding the others, more definite driving variables can be identified the more the decomposition proceeds.

In the soil (Fig. 4-20), it is shown that the summer points of both locations are differentiated from the autumn/spring ones, yet in a less sharp way than in the litter. The 0-5 cm is the most complex to interpret and only 30% of the variance is explained in the first principal component. Summer points are discriminated by cellulase and xylanase activity and for the lignin content, and the summer points for Pradaccio fall in the opposite quadrant of the PCA compared to the other autumn/spring points of the same location. As a matter of fact, while we can see a clear difference between autumn/spring samples of Laceno and Pradaccio mostly due to the different C:N ratio, in the 0-5 cm layer the samples of Pradaccio's summer get closer to Laceno's ones N content, dehydrogenase and laccase activities. In the deeper layers of the soil, Laceno and Pradaccio's point return to occupy different quadrants in the scree plot, with the summer points discriminated mostly by N content, acid phosphomonoesterase, cellulase and xylanase. The variables which justify the differences between the locations are the organic matter, cellulose and lignin contents plus the C:N ratio. As for the litter, we can detect that, the deeper the soil, the greater the variance expressed by the first principal component (from 30% to 44%), with some variables (e.g. C:N ratio, cellulase, xylanase, N) getting more important and others (e.g. dehydrogenase and peroxidase) reducing their impact.

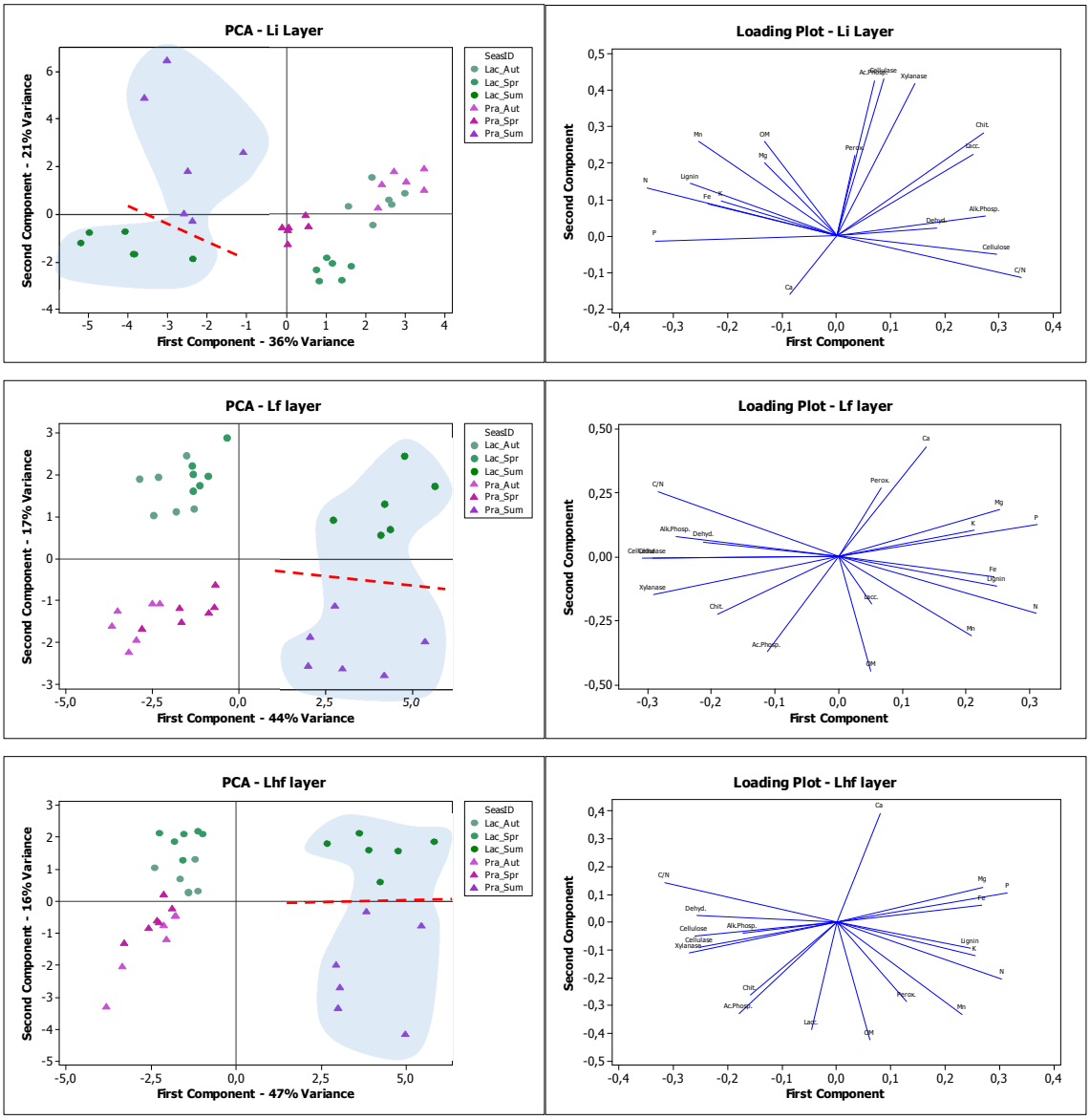


Figure 4-19: Principal Component Analyses (on the left side) and corresponding loading plots (on the right side) for the three layers of the leaf litter decomposition continuum of the seasonal sampling. The shaded areas identify the summer sampling and the red lines divide Laceno from Pradaccio.

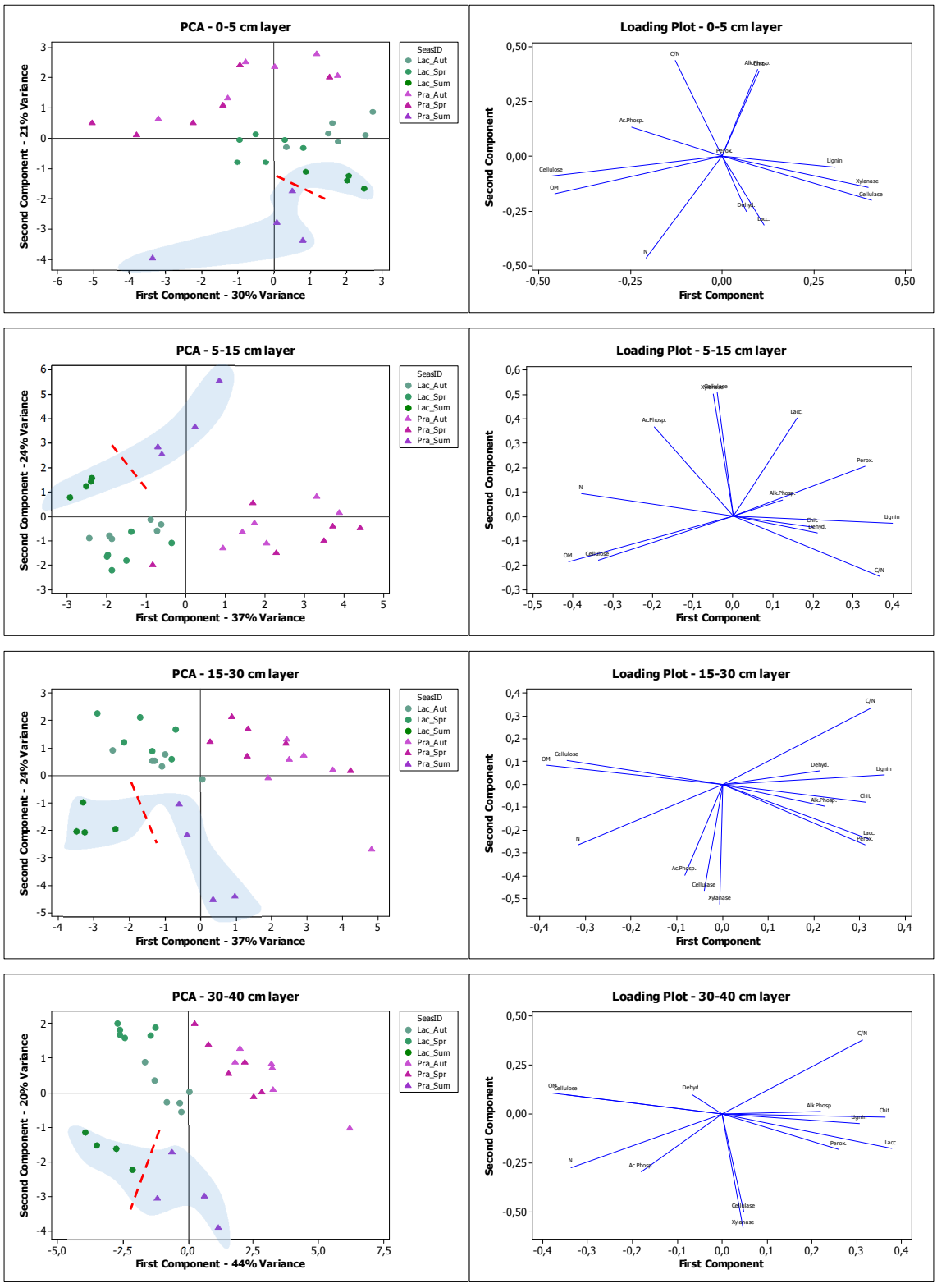


Figure 4-20: Principal Component Analyses (on the left side) and corresponding loading plots (on the right side) for the four layers of the soil of the seasonal sampling. The shaded areas identify the summer sampling and the red lines divide Laceno from Pradaccio.

4.5 Discussion

The results from this section of this research overlap and complete those of the first section. Whereas the litterbag experiment allowed to have an insight on the decomposition processes in the two studied locations and to assess the impact of plant/soil quality and climate during the decay, the seasonal analysis allows to broaden the view. First of all, the litter coming from the forest floor gives a wider picture of the real overall microbial community in the locations. Even the Li layer is a combination of freshly fallen plant material and more decomposed material (Sjöberg et al., 2004) and the differences of microbial communities that can be in the decomposition continuum may be astonishing even within a layer 1 cm thick (Šnajdr et al., 2008a).

The results showed a sensible reduction of organic matter (and thus C and N) along the decomposition continuum. The relationship between the concentration of C and cellulose and N with lignin suggest the trends of decomposability/recalcitrance of this compounds along the decomposition continuum and along depth. Accordingly, recalcitrance of soil organic carbon decreases with depth, while in contrast the recalcitrance of nitrogen is roughly maintained with depth (Rovira and Vallejo, 2007; Rovira et al., 2008). Similar trend in the content of cellulose and lignin, mostly for the litter or organic layers, are known from other studies (e.g. Donnelly et al., 1990; Fioretto et al., 2005; Romaní et al., 2006; Sjöberg et al., 2004; Ververis et al., 2007).

Figure 4-21: Differences in the soil cores between Pradaccio (right) and Laceno (left). Pictures are not in scale and the orange arrow point to the 0-5 cm layer. Pictures by Prof. Dr. Antonietta Fioretto (2011).

The content of organic matter is shown to be much higher in Pradaccio than Laceno in the uppermost layer, but higher in Laceno in the deeper layers. Litter quality is higher in Pradaccio, and also soil quality appears to be higher, as shown in the litterbags laboratory experiment. Thus, even though on the field the decomposition is faster in Laceno, there is a higher concentration of organic matter in the soil. The sharp difference in the distribution of organic matter along the depth between the two locations was already evident since the opening of the soil cores (Fig. 4-21). The explanation could be derived to the higher annual litter input of Laceno, due to the longer vegetative season which allows to grow larger leaves and in greater quantity. An alternative or complementary explanation

could derive by different times necessary to reach a stable or very slowly decomposed fraction in the decomposition of the organic matter, which is a parameter which was not possible to evaluate within the timeframe of the presented experiments. These times are shown to be extremely variable and greatly impacting the accumulation of organic matter (Berg and McClaugherty, 2014).

Figure 4-22: Morphological differences between the leaf litter of Pradaccio (left) and Laceno (right). Pictures are in scale. Image scan by Agostino Bortone M.Sc. (2012).

A morphological survey on the leaves of Laceno and Pradaccio (and other beech forests from the Apennines) has been done, but the results are not presented in this work and they will be included in a separate manuscript (Fig. 4-22). Preliminary results from Laceno show that the leaves of the Li layer of the litter in the southern site are larger (20 cm^2 vs. 14 cm^2 of Pradaccio), that they are heavier (110 vs. 100 cm^2 for Pradaccio) but that they have a lower leaf mass per area (6 vs. 7 mg/cm^2 for Pradaccio). Leaf mass per area has been shown to be higher in dry locations but also in cold locations, as it is a diversified response of the plant to stress factors (Ogaya and Peñuelas, 2007).

Another factor related to the longer growth season and the milder climate of Laceno could be the greater presence of the undergrowth. Although, as for many beech forests, there is not a very large understory vegetation, Laceno has more shrubs and herbs than Pradaccio. Dwarf shrubs produce litter rich in lignin and phenols (Vargas et al., 2006) and the slow decomposability of dominant vegetation is thus reflected in the soil organic matter in the late stage of decomposition and higher concentrations deeper in the soil (Hilli et al., 2008).

Moreover, the analysis along the depth of the soil allowed to add information which was not available in the decomposition experiment, both in terms of quantity/quality of the organic matter

and microbial activities. Tate (1979) observed that microbial activity of the soil generally decreased with increasing depth. That indicated that the total carbon evolved from the different levels of the soil profile by the microbial community oxidizing the soil organic matter decreased as the depth of the soil column increased. However, when correcting for the amount of carbon yielded at each level for the bulk density of that level revealed that the microbial contribution to the soil subsidence is approximately equivalent throughout the soil profile. The results of this study agree to these findings, being the microbial activities even higher deeper in the soil in comparison to the abundance of organic matter. For some very specific C sources, as lignin-like substances, the activity of laccase and/or peroxidase increased strongly in deeper layers of the soil, suggesting the presence of differentiated and specialised fungal communities.

The analysis of microbial activity and organic matter in the deeper layer of the soil is not as known as the upper layers (Houghton, 2003). The densities of microorganisms is generally one to two orders of magnitude lower in the deeper horizons than at the soil surface and approximately 35% of the total quantity of microbial biomass in the top 2 m is found within 0 and 25 cm (Fierer et al., 2003). Besides, there is a great change of microbial communities along the soil profile, with Gram-positive bacteria and actinomycetes tending to increase in proportional abundance with increasing soil depth, while the abundances of Gram-negative bacteria, fungi, and protozoa is highest at the soil surface and substantially lower in the subsurface. The vertical distribution of these specific microbial groups can largely be attributed to the decline in carbon availability with soil depth (Fierer et al., 2003).

The trend of enzyme activities has already been shown that, especially in Mediterranean ecosystems, they have great diversity with seasons, being lower during the arid times of summer or the coldest moment of winter (Fioretto et al., 2009, 2005b; Papa et al., 2008; Sardans and Peñuelas, 2005). On *Quercus petraea* forest soil, Šnajdr et al. (2008b) found that activities of all tested enzymes exhibited high spatial variability in the L (largely corresponding to the Li layer of this study) and H horizons (largely corresponding to Lhf plus parts of 0-5 cm soil layer). In detail, acid phosphatase and 1,4-b-N-acetylglucosaminidase (chitinase) exhibited low variability in both horizons, while the variability of Mn-peroxidase activity in the L horizon, and endoxylanase and cellobio-hydrolase activities in the H horizon were very high (Šnajdr et al., 2008b).

Dehydrogenase, as index of total microbial activity, has been shown to be almost double in autumn than in summer for forest soils and that the enzyme activity was largely dependent on pH (Quilchano and Marañón, 2002). These findings match those of this thesis, with dehydrogenase activities generally higher in autumn-spring (for Laceno) and spring-summer (for Pradaccio), and the differences in absolute values could be also explained according to the different pH.

The patterns of changes in litter quality reflect what happens in the litterbags, but some increases (e.g. the increase of N and Fe) and much broader than what happens in the litter bags. The phenomenon has been shown to be more extensive for the summer season in both locations, although with the differences in absolute and relative concentration which was already seen in the litterbags.

The differences in the values could be easily explained by the filter effect of the litterbags which, even though are an extremely good way to assess decomposition, represent a non-natural condition. Besides, the mesh size used in the decomposition experiment did not allow the access to all of the soil fauna. The impact of fauna, especially Collembola and Anellida, is substantial in translocating elements from the deeper layers of the soil to the surface and vice versa (Andriuzzi et al., 2013; Chamberlain et al., 2006; Cortez and Bouché, 1998) and some species of earthworms, which are too large to penetrate the mesh size of the used litterbags, are specially responsible of a sensible increase of N and Fe (Cortez and Bouché, 2001, 1998). The impact of soil fauna contributes so largely to soil phenomena that field experiments using insecticides showed that excluding micro arthropods reduced rates of forest litter decomposition (Blair et al., 1992). The activity of soil fauna could be also an explanation for the increase of organic matter and the changes of C:N ratio during summer. Total soil carbon storage has been shown to be positively and significantly correlated with earthworm density and occurrence of earthworm cast in topsoil, which indicated that bioturbation could play an important role in soil carbon storage and mobilization (Frouz et al., 2009).

The principal component analysis, for both litter and soil, always confirmed the greater diversity of the summer season compared to the spring/autumn. Biplots clarified that, for litter, summer showed a change in all the parameters of the litter quality while autumn/spring had more diversity for the enzyme activities. In contrast, in the soil, a set of enzyme activities contributed to explain the greater diversity of the summer season, even deeper in the soil. Differences could be appreciated also between the locations, with a shifting of some enzymes activities between Laceno and Pradaccio.

Some of my results are comparable to the study of Blume et al. (2002), concerning the microbial activities in two soil both planted at corn, and the difference between the land use has to be stressed. The results from the cited study were that the size of the microbial biomass in both the surface and the subsurface soils was not significantly affected by the seasonal variation, but activity increased by as much as 83% at the summer temperatures in the surface soil and that for both soils seasonal changes in the subsurface microbial community occurred. Their results suggested that winter conditions will shift the population activity level in both the surface and subsurface systems and the biochemical structure of the community in the subsurface. These findings can partly overlay to the forest ecosystems that have analysed in the discussed research but, in contrat, the authors of the cited study found that the inorganic chemical properties of the soil, as a function of season, remained constant (Blume et al., 2002).

The impact of climate between Laceno and Pradaccio has already been discussed. This second section of the results concerning these two locations contribute in closing a general picture of what happens from the litter until 40 cm depth in the soil. Both locations clearly showed an increase in nutrient contents in the decomposition continuum of the litter during summer, which could be explained by the increase in soil fauna activities. The activities of earthworms contributes in retranslocating organic matter in available forms from the upper layers to the deeper layers,

enhancing the microbial enzyme activities even in deeper layers of the soil. In the litter (and partly in the soil), enzyme activities appear to be generally higher in Laceno during autumn and spring, while for Pradaccio the peak is generally more shifted to the summer period, although some activities were relevant also in autumn. This evidence can be supported by the already mentioned climatic differences and the litterbags experiment, and the drought summer periods (more evident in Laceno) appear to be the greater factor slowing down the microbial activity. For Pradaccio, the limiting factor is likely the colder climate and the snow cover, with the whole soil phenomena more concentrated between May-October, with summers as a particularly positive time of the year.

Results achieved in these years, but not shown in this work (as they are still under development and expansion), show a different pattern of isoenzymes of laccase and peroxidase between the two locations, in the litter layers and between the seasons. The results concur to the general assessment of this thesis of a different fungal community between Laceno and Pradaccio and, where the isoenzymes where similar, they could be found into different layers of the decomposition continuum according to season and location. So far, a similar approach has been done only for *Quercus ilex* litter (Di Nardo et al., 2004).

Further investigation on the microbial community, their succession and their response to ecological changes (with experiment into microcosms) will contribute to further understand the dynamics of enzyme activities in the litter and soil of forests. The results could be also related and expanded with the analysis on Mn and its importance in the decomposition dynamics, as already shown in the first part of the results.

5 Results from the north-south Apennine transect

Peak of Mt. Raiamagra (1700 m) in winter, close to Laceno. The lower temperatures and the strong winds allow the beech to grow only in a shrub-like morph (specimens on the right of the picture).

5.1 Standing leaf litter and soil bulk density

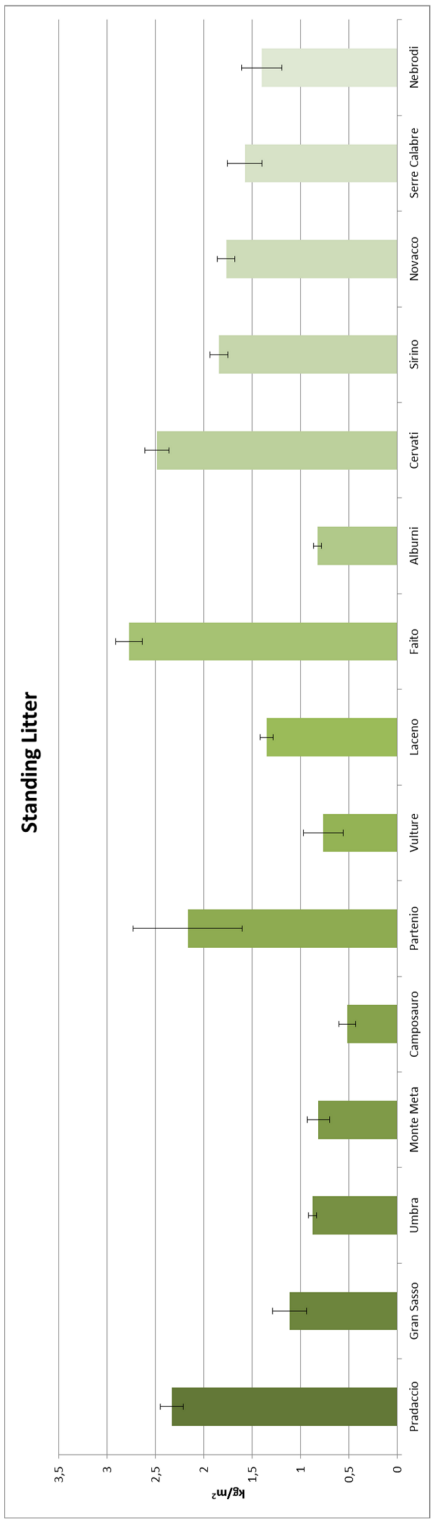


Figure 5-1: Standing litter in the 15 locations of the north-south Apennine transect. Values are mean±s.e. (n=3).

The amount of standing leaf litter, meant as the sum of the masses of Li, Lf and Lhf per square meter, showed a great variability among the locations (Fig. 5-1). The greatest values were recorded in Faito, Cervati, Pradaccio and Partenio (2.8, 2.5, 2.3 and 2.2 kg/m^2 respectively), while the lowest values were those of Camposauro and Vulture (0.5 and 0.8 kg/m^2).

The bulk density of the soils generally increased along the depth from 0-5 to 30-40 cm (Fig. 5-2). The median value for the 0-5 cm layer was 0.6 g/cm^3 while for the 30-40 cm layer it was 1.1 g/cm^3. In some locations, the increase of bulk density with depth was much evident (e.g. Pradaccio, where the bulk density in the deeper layer was 4 times higher than the upper layer) while in other locations (Camposauro and Laceno) this increase was less sharp or even not present.

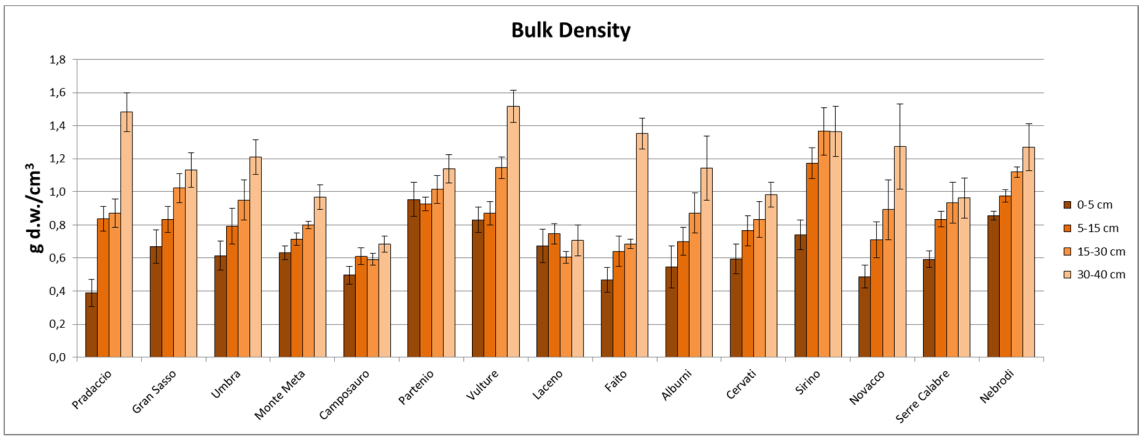


Figure 5-2: Bulk densities in the soil layers of the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

5.2 Quantity and quality of organic matter along the Apennine profile

The concentration of organic matter (O.M.) along the decomposition continuum of the litter decreases from the Li to the Lhf layer in all studied locations (Fig. 5-3). The overall median concentration of O.M. in the decomposition layer was 93% for Li layer (95% confidence interval was 91-94%), 91% for Lf layer (95% c.i. was 90-92%) and 86% for Lhf layer (95% c.i. was 79-88%). Generally, there is a much more evident drop in O.M. content in Lhf layer compared to the other layers. Sometimes, this fall is much evident (e.g. Umbra, from 85% O.M. in the Lf layer to 78% in the Lhf layer or Nebrodi, from 90% to 75% etc.). In other cases, this reduction is less sharp (e.g. Pradaccio, Gran Sasso, Faito etc.). An exception to the general concentration of O.M. are the locations Partenio and Vulture. In detail, in Partenio the concentration of O.M. is, even from the Li layer, generally much lower than the average concentration of the Lhf layer from the other locations. In Vulture, the Lhf layer was scarcely present, thus the absence of a clear error bar in the graph.

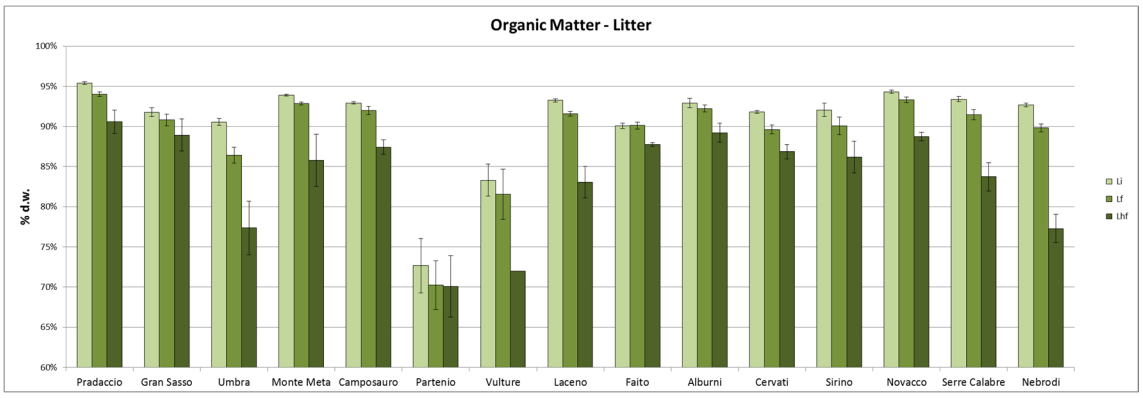


Figure 5-3: Concentration of O.M. in the leaf litter decomposition continuum for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

In the soil (Fig. 5-4), there was a general trend to a decrease of O.M. from the 0-5 cm layer to the 30-40 cm layer. The overall median concentration of O.M. was 24% in the 0-5 cm layer (95% c.i. was 20-32%), 16% for the 5-15 cm layer (95% c.i. was 13-20%), 12% for the 15-30% layer (95% c.i. was 9-18%) and 9% for the 30-40 cm layer (95% c.i. was 8-13%). Usually, as could be expected, the 0-5 cm layer was the richest in O.M and, normally, a sharp decrease was recorded in the deeper layers. Sometimes this reduction was not so severe (e.g. Partenio or Laceno) while in other cases it was particularly intense (e.g. Pradaccio, Faito or Novacco). In Laceno, as already observed, the concentration of O.M. slightly increased from the 15-30 cm to the 30-40 cm layer.

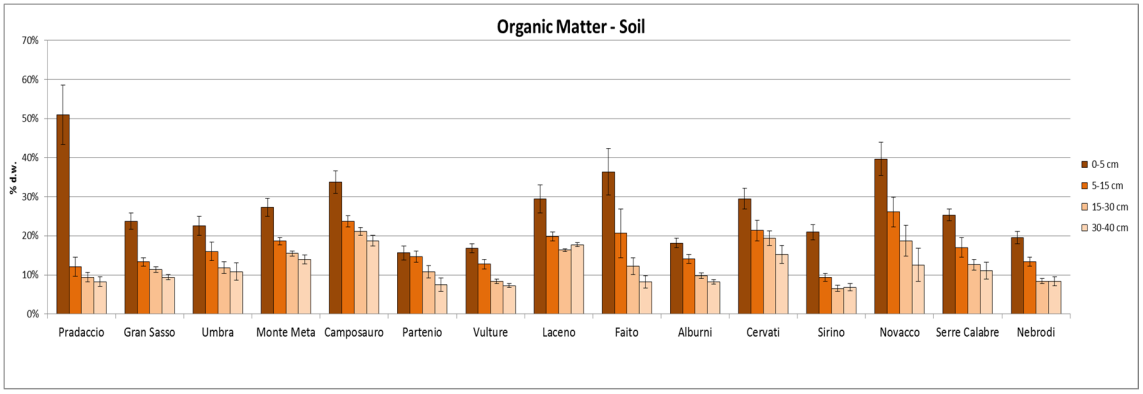


Figure 5-4: Concentration of O.M. in the soil for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

On a mass per area basis, considering the standing leaf litter and taken into accounts the different bulk densities of the soils' layers and their respective length, the total bulk of O.M. can be appreciated from the standing litter to 40 cm (Fig. 5-5). The standing litter had usually much lower mass of O.M per square meter, being on average 1.3±0.16 kg/m^2. The highest values can be seen in Faito and Cervati (2.5 kg/m^2 each) and the lowest are in Camposauro (0.46 kg/m^2). In the soil, the greatest bulk of the whole soil length (from 0 to 40 cm) was found in Cervati, Laceno, Camposauro and Monte Meta (55, 52, 49 and 47 kg/m^2 respectively), while the locations that showed the lowest bulk were Sirino, Faito, Umbra and Pradaccio (19, 23, 27 and 27 kg/m^2 respectively).

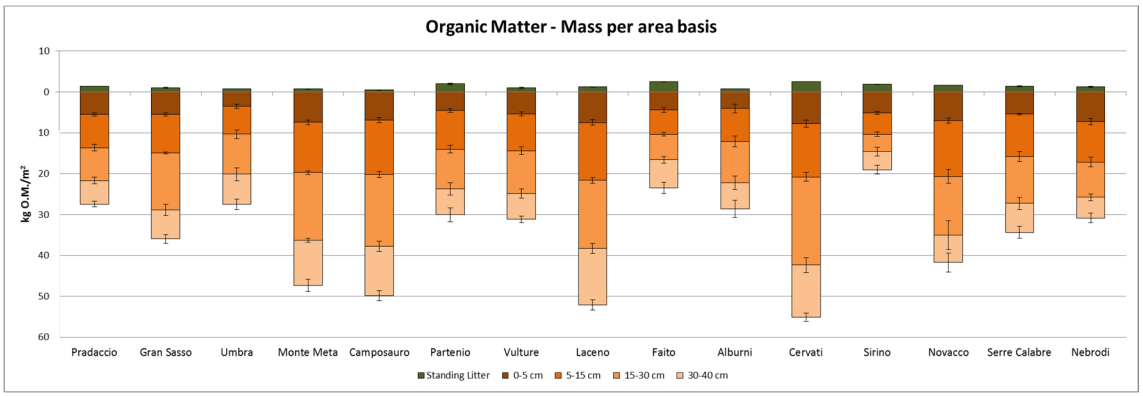


Figure 5-5: Organic Matter expressed on mass per area basis for both standing litter and soil for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

The trends of C, both in the litter and soil, closely followed the trends of the O.M. According to the linear relationship existing between the two variables (Fig. 5-6), a factor of 2 can be adopted to pass from O.M. to C and vice versa, especially in the soil.

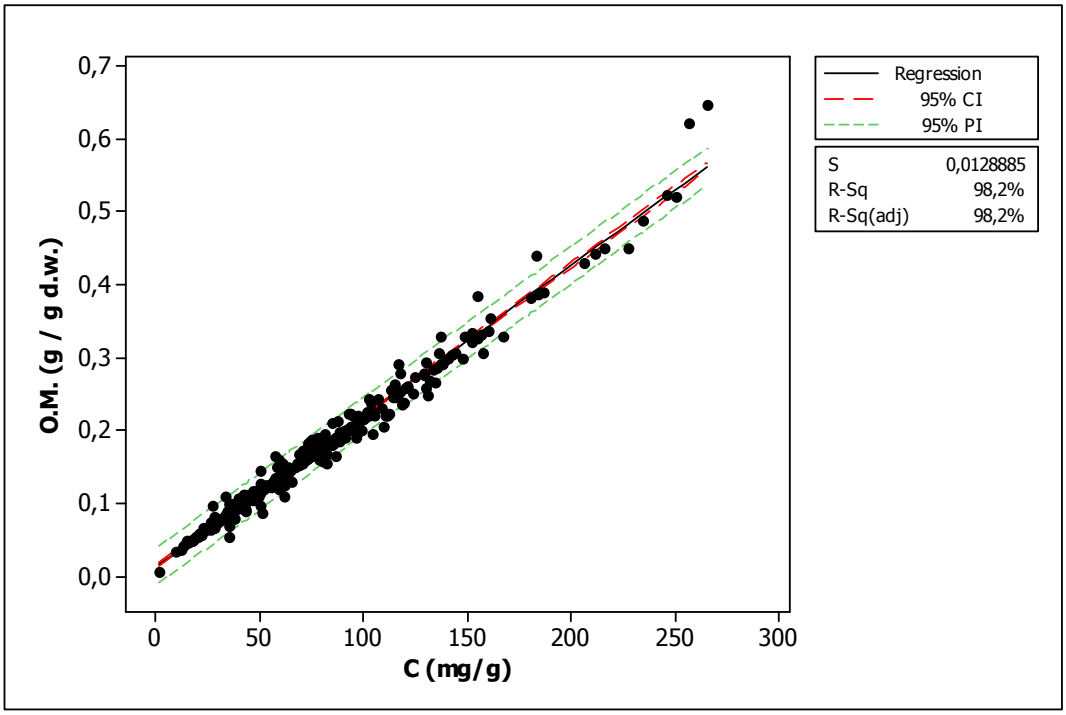


Figure 5-6: Relationship between organic matter and C in the soil. C is usually ½ O.M.

Only for comparison with other studies related to the bulk amount of C in the soil, the graph on mass per area basis is shown (Fig. 5-7). The results, neat of some minor differences, are overlying those of the organic matter.

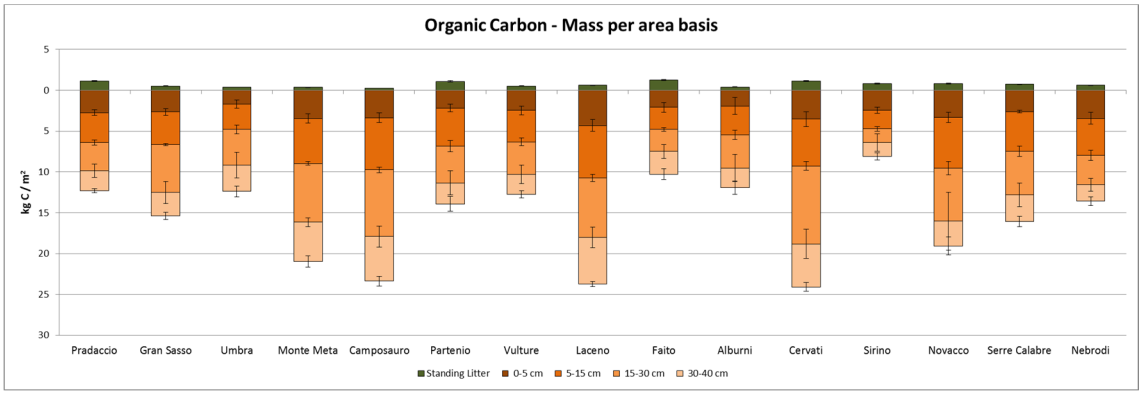


Figure 5-7: Carbon expressed on mass per area basis for both standing litter and soil for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

Nitrogen in the litter showed a general tendency to increase along the decomposition continuum, as already previously shown (Fig. 5-8). The median values for the Li, Lf and Lhf layer were 16, 19 and 21 mg/g respectively. In general, N was immobilized along the decomposition continuum with

a 1.3 factor from the Li to the Lhf layer, although in some cases, like Pradaccio, the increase was much higher (1.8 folds increase) while in other locations the increase was less evident. A few locations (Gran Sasso, Partenio and Vulture) showed a different trend, with a non-linear function of release/immobilization of N along the decomposition continuum. For Vulture, as already mentioned, the Lhf layer was not easily identifiable, thus its N value cannot be estimated as fully reliable.

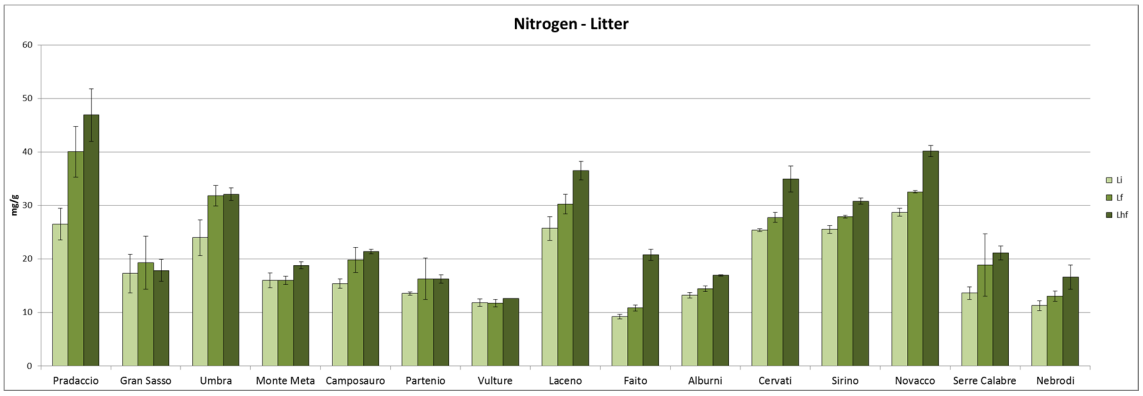


Figure 5-8: Concentration of nitrogen in the leaf litter decomposition continuum for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

In the soil (Fig. 5-9), a sharp decrease can be seen along the soil depth in all locations. The highest values of N were always in the 0-5 cm layer, as expected from the higher O.M. content, and had a median value of 11 mg/g. The locations with the average higher concentrations of N in the 0-5 layer were Novacco, Faito and Cervati (24, 19 and 16 mg/g respectively) while those with the lower values were Partenio, Laceno and Nebrodi (6, 7 and 8 mg/g respectively). The deeper layers (5-15, 15-30 and 30-40 cm) had median values of 6, 4 and 3 respectively. Thus, generally N decreased by a factor of 3 from the 0-5 cm to the 30-40 cm layers. Considering the average values for the whole soil length (from 0 to 40 cm), the higher values were shown by Novacco and Cervati (14 and 10 mg/g respectively), while the locations with the lower concentrations were Partenio and Pradaccio (5 mg/g each).

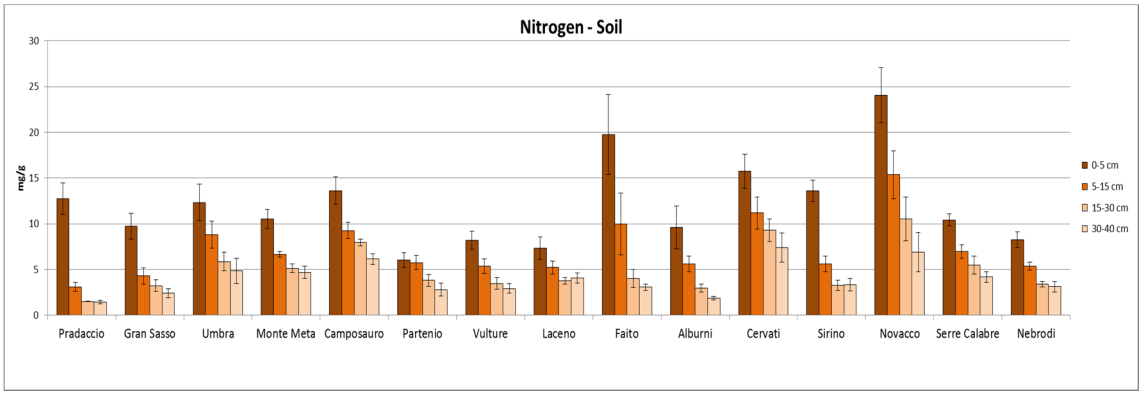


Figure 5-9: Concentration of nitrogen in the soil for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

Considering the differences in N from the litter Lhf layer to the soil 0-5 cm layer, generally a decrease of 50% can be noticed (Fig. 5-10). Some locations are very much above this value (e.g. Laceno and Pradaccio with 79 and 72% nitrogen lost from the Lhf to the 0-5 cm layer), many others are around of slightly below the average value (e.g. Alburni, Vulture, Monte Meta etc.) while in Faito there is almost no difference between N in the Lhf and that in the 0-5 cm layer of the soil.

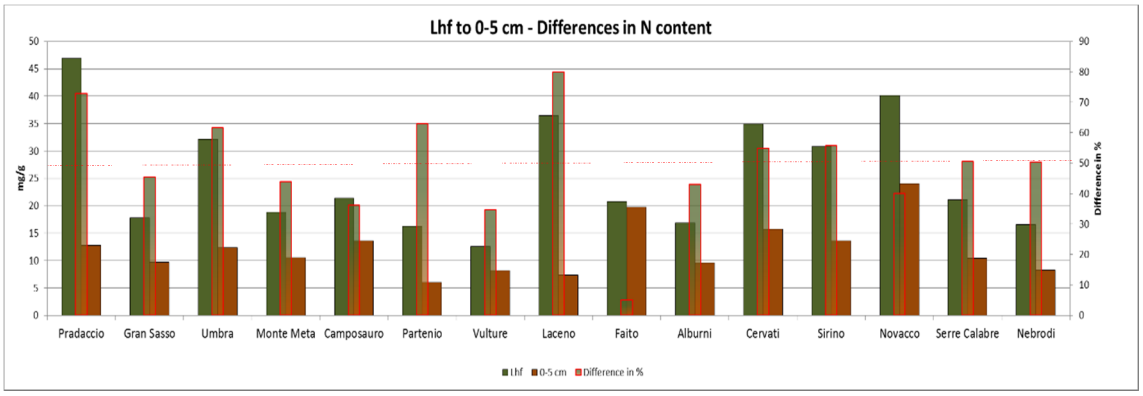


Figure 5-10: Differences in N between the litter Lhf layer and the soil 0-5 cm layer. The middle column with the red outlines represents the difference in %. The red dotted line represent the 50% difference.

On a mass per area basis (Fig. 5-11), the concentration of N in the standing litter was on average 30±5 g N on square meter, with maximum values for Pradaccio and Cervati (88 and 73 g/m^2 respectively) and the lower values were for Alburni and Camposauro (12 and 13 g/m^2 respectively). In the soil, the greatest bulk (from 0 to 40 cm) can be recorded in Cervati and Novacco (2.7 and 2.4 kg/m^2 respectively) and the lower ones for Pradaccio and Alburni (0.7 and 0.9 kg/m^2 respectively).

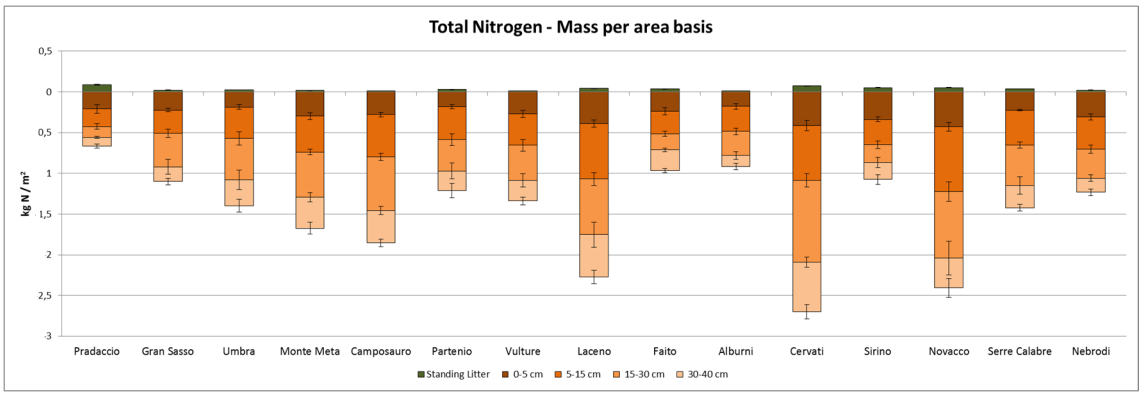


Figure 5-11: Nitrogen expressed on mass per area basis for both standing litter and soil for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

The carbon to nitrogen ratio in the litter reflected, as predictable, the trends of the two variables along the decomposition continuum (Fig. 5-12). Generally, a decrease from the Li to Lhf layer was seen in all locations, with the exception of Gran Sasso where this decrease was not so sharp. The higher values of C:N in the Li layer were those of Faito and Nebrodi (51 and 45) while the lower ones were Novacco and Cervati (17 and 18). In the Lhf layer, the highest values were Vulture and Gran Sasso (31 and 27) and the lowest ones were Pradaccio and Novacco (9 and 10). The sharpest

relative decrease of the C:N ratio in the litter was seen in Pradaccio and Faito, where the ratio in the Lhf layer was 52 and 55% of the ratio in the Li layer, respectively. The slowest relative decrease, instead, was shown in Gran Sasso and Vulture, where the ratio in the Lhf layer was correspondingly 93 and 83% of the Li layer.

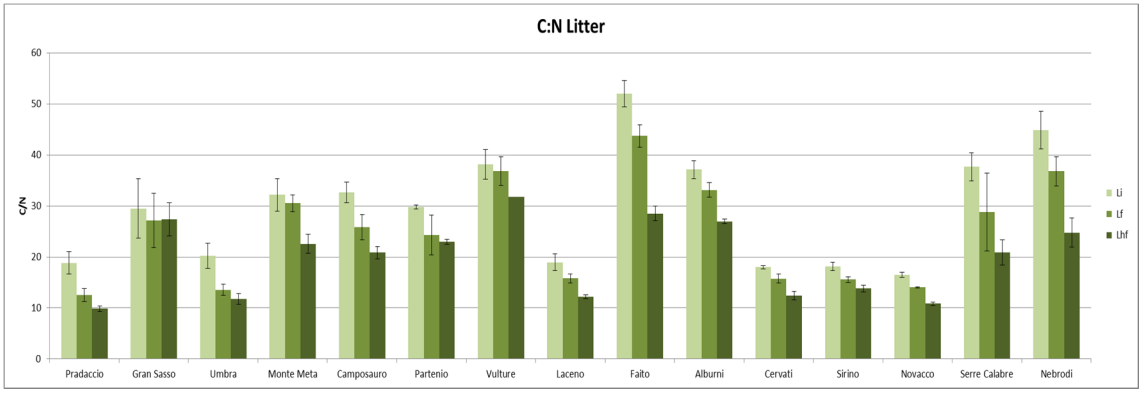


Figure 5-12 C:N ratio in the leaf litter decomposition continuum for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

The trend of C:N in the soil followed a more complex pattern (Fig. 5-12). Generally, the values were always between 10-15. Many locations exhibit an increase from the 0-5 cm to the 30-40 cm layer (e.g. Umbra, Monte Meta, Vulture, Alburni etc.) while other locations show a certain constancy in the values (e.g. Pradaccio, Camposauro, Cervati, Novacco etc.). A conspicuous exception is Gran Sasso, where there is a great variability in the ratio in the deeper layers of the soil, especially between 5 and 30 cm depth.

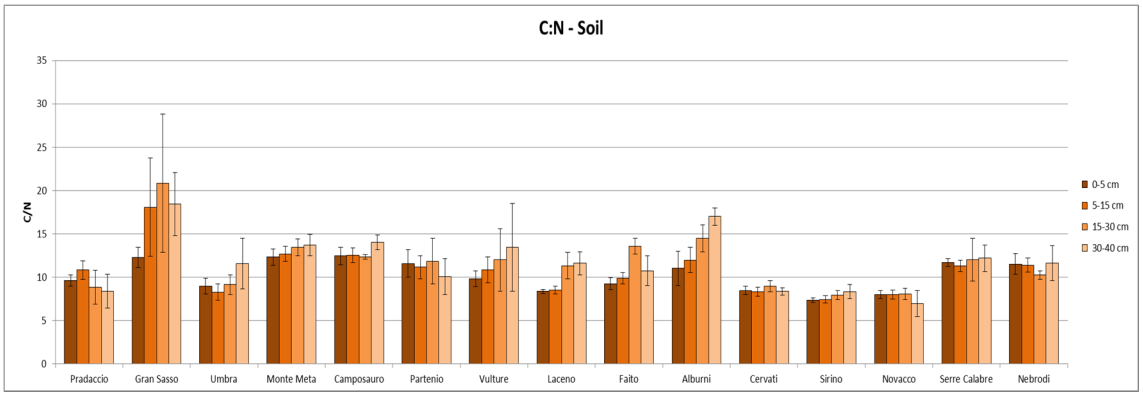


Figure 5-13 C:N ratio in the soil for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

As concerns cellulose and lignin, it is worth to remind once again that the values are expressed as function of the organic matter content, thus their actual contribute on a dry matter basis can be highly diverse according to the concentration of O.M. For the litter, generally, there is decrease of cellulose and an increase of lignin the further we progress on the decomposition continuum (Fig. 5-14). Cellulose and lignin had median values in the Li layer of 32 and 45% of the organic matter respectively, while in the Lhf layer the median values are 25% of cellulose and 55% of lignin in the organic matter.

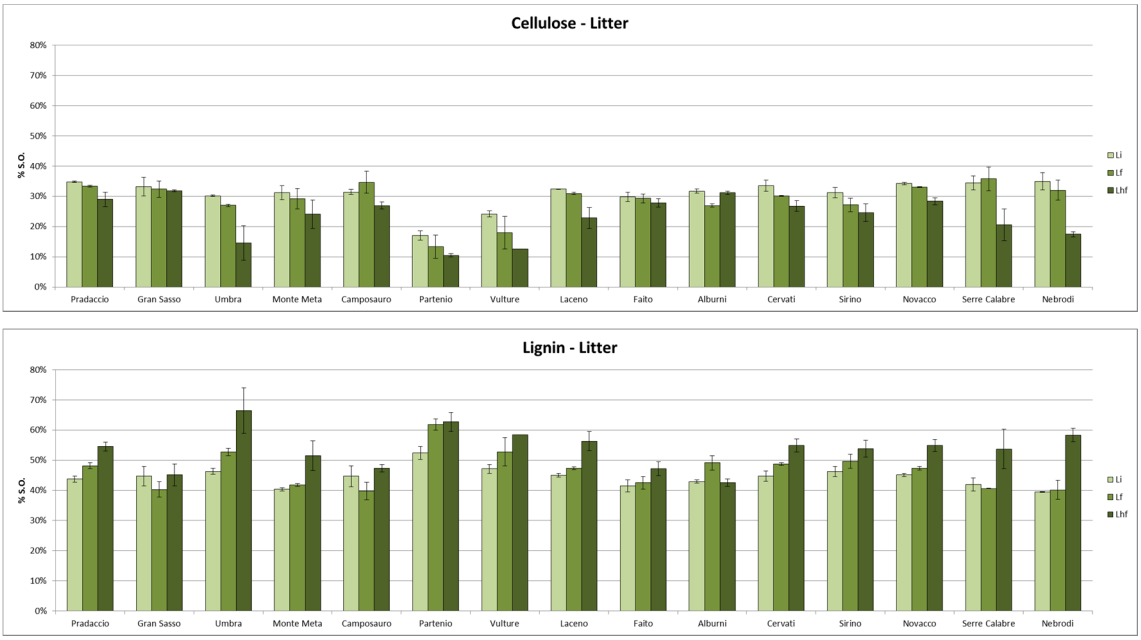


Figure 5-14: Concentration of cellulose (above) and lignin (below) in the leaf litter decomposition continuum for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

In the litter, cellulose clearly shows a trend related to the content of C according to a second order polynomial regression with an excellent prediction power (R^2adj.=0.803). Lignin, instead is linearly related to the content of N, although the R^2adj. of the regression is lower (0.685) (Fig. 5-15).

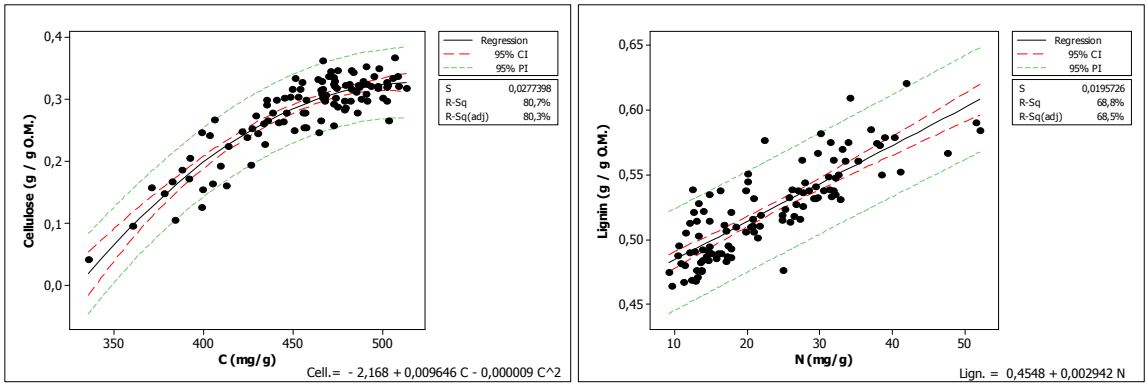


Figure 5-15: Polynomial (2nd order) regression with cellulose as dependent variable and C as independent variable (left) and linear regression between lignin as dependent variable and N as independent variable (right) in the leaf litter decomposition continuum. Red lines represent 95% confidence interval while green lines represent 95% prediction interval.

As for the soil (Fig. 5-16), it is important to pay attention to the different *y* scale in the figures reporting the concentration of cellulose and lignin as a function of the organic matter. Accordingly, cellulose is always a very low percentage of the organic matter, and it was usually below 5% after 5 cm of depth. Only the 0-5 cm layers of some locations (i.e. Laceno, Faito, Alburni, Sirino and Novacco) had more than 8% of cellulose in their O.M. with the noticeable exception of Pradaccio where 20% of the O.M. is cellulose. Generally, a very sharp decrease of its content is seen from the uppermost layer. On the contrary, lignin is clearly the most abundant fraction of the O.M. in the soil and its contribute increases the deeper the soil. Laceno has the lowest concentration of lignin in its

soil organic matter but higher concentrations of cellulose. Lignin is in most cases shown to be more than 50% of the O.M. and sometimes, in the deeper layers, it can be between 80 and 90% of the O.M.

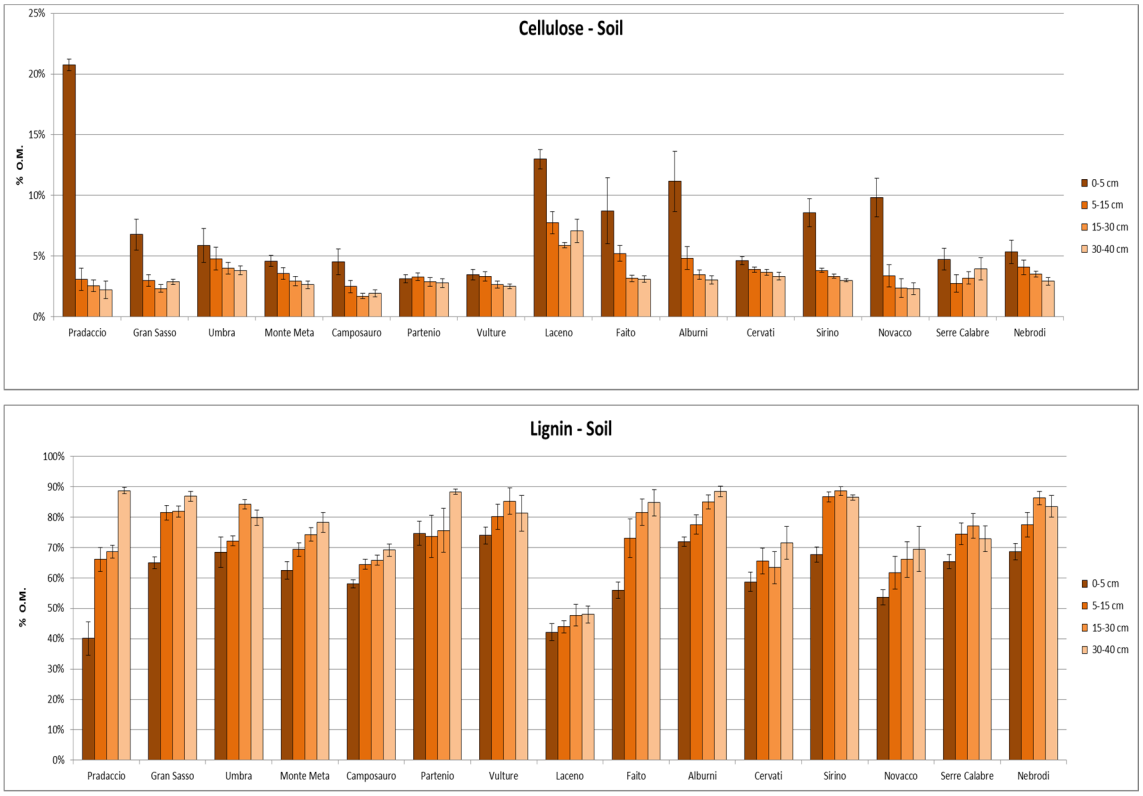


Figure 5-16: Concentration of cellulose (above) and lignin (below) in the soil for the locations in the north-south Apennine transect. Values are mean±s.e. (n=6).

As for the litter, cellulose and lignin in the soil are strongly correlated to the concentration of C and N, respectively. However, in contrast with the litter, the relationship is stronger for lignin and N than for cellulose and C (R^2adj 0.671 vs. 0.353) and, whereas in the litter both relationship responded to a positive correlation, in the soil the correlation for lignin and N is negative (Fig. 5-17).

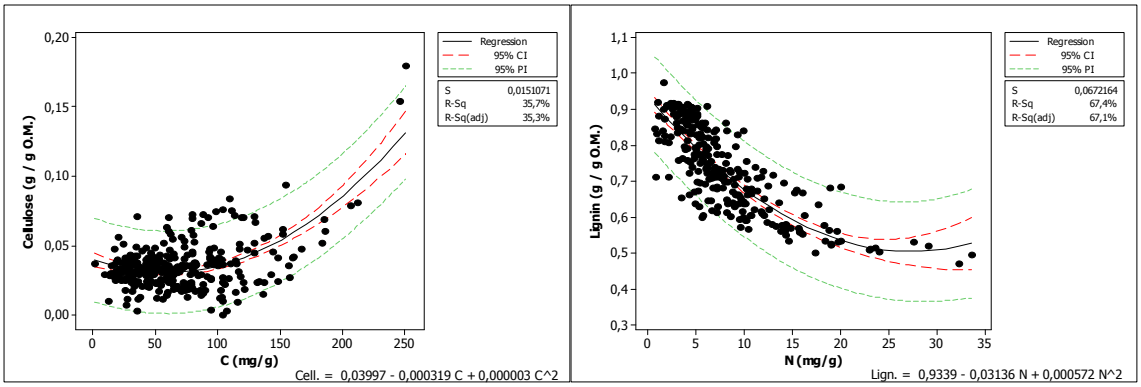


Figure 5-17: Polynomial (2nd order) regression with cellulose as dependent variable and C as independent variable (left) and polynomial (2nd order) regression between lignin as dependent variable and N as independent variable (right) in the soil. Red lines represent 95% confidence interval while green lines represent 95% prediction interval.

5.3 Relationships between O.M. and ecological variables and statistical modelling

Table 5-1: Spearman correlation coefficients between ecological variables and content of O.M. in the 0-5 cm soil layer and the mean content of O.M. in the leaf litter. Values in bold are statistically significant.

	Content of O.M. in 0-5 cm soil layer	Mean content of O.M. in leaf litter
Latitude (degree)	0,118	0,100
Longitude (degree)	-0,236	-0,229
Elevation (m amsl)	0,307	0,479
Asperity Index	0,004	-0,236
Aspect (degree)	0,114	0,164
Mean Annual T (°C)	-0,449	**-0,642**
Isothermality	-0,376	-0,369
Max T of Warmest Quarter (°C)	**-0,528**	**-0,716**
Mean T of Driest Quarter (°C)	-0,507	**-0,707**
Mean Annual Precipitation (mm)	**0,577**	**0,720**
Precipitation of Warmest Quarter (mm)	**0,588**	**0,690**

To assess the impact of ecological variables on the accumulation of organic matter, a Spearman rank correlation analysis has been done. The content of O.M. in the 0-5 cm soil layer (the layer with the highest relative concentration of O.M.) and the mean content of O.M. in the leaf litter decomposition continuum (i.e. the mean of the three layers Li, Lf and Lhf) have been compared to a set of ecological variables derived from the WorldClim dataset (Hijmans et al., 2005) (Tab. 5-1). Five ecological variables relate to geography and topography (i.e. latitude, longitude, altitude, asperity index and aspect) and none of them appeared to be correlated to neither of the O.M. contents. Four other variables are connected with temperatures (mean annual temperature, isothermality, maximum temperature of the warmest quarter and mean temperature of the driest quarter) and, generally, there was negative correlations with the O.M. contents, especially for the litter. In detail, the highest and most significant correlations were between the contents of O.M. and the maximum temperature of the warmest quarter. The last two ecological variables are about precipitation regimes (mean annual precipitation and precipitation of the warmest quarter) and, in this case, positive correlations could be found, once again more significant for the litter than for the soil. Thus, a complex interaction between temperatures (which has a negative effect) and rainfall (which has a positive effect) regulates the accumulation of organic matter in the leaf litter and, secondarily, in the top layer of the soil.

Several modelling approaches to describe the accumulation of the organic matter as a multiple linear combination of the ecological variables have been attempted, but none of them had any reliable result. The results, when a multiple linear regression was possible and providing statistically significant relationship (although with a very low R^2adj.), were always limited to 0-5 cm layer only. Although linear models should always be preferable to polynomial models, even when they provide

lower determination coefficients, several polynomial models attempting to find a relationship which could be stable for all layers have been tested (Griepentrog et al., 1982).

Despite the absence of a significant correlation, the variable altitude appeared to be the more interesting for a statistical modelling approach. In fact, altitude is a crucial factor in regulating temperature and precipitation regimes in mountain ecosystems. Altitude and the concentration of O.M. (for all layers, in contrast to multiple linear models) could be related by a third degree polynomial regression with a very high R^2(adj.), considering that the amount of observations is limited (Fig. 5-18). In figure, the relationship between the upper layer (0-5 cm) and the altitude is shown. The model suggests that, between 1000 and 1350 m, there is an increase of O.M. in the soil and, after 1350 until 1600 m, a decrease. The model is also able to explain the concentration of O.M. in the heterotopic stands (Vulture and Umbra). Elevation, as a single variable, explains the complex interactions of precipitation and temperatures that regulate complex ecological phenomena such as primary production, decomposition, soil biota, evapotranspiration, soil respiration etc. The heterotopic stands are located at elevations that are below the normal minimum level of the beech in the Italian Apennines (i.e. ≈800 m), but mesoclimatic or microclimatic conditions compensate the lower elevation.

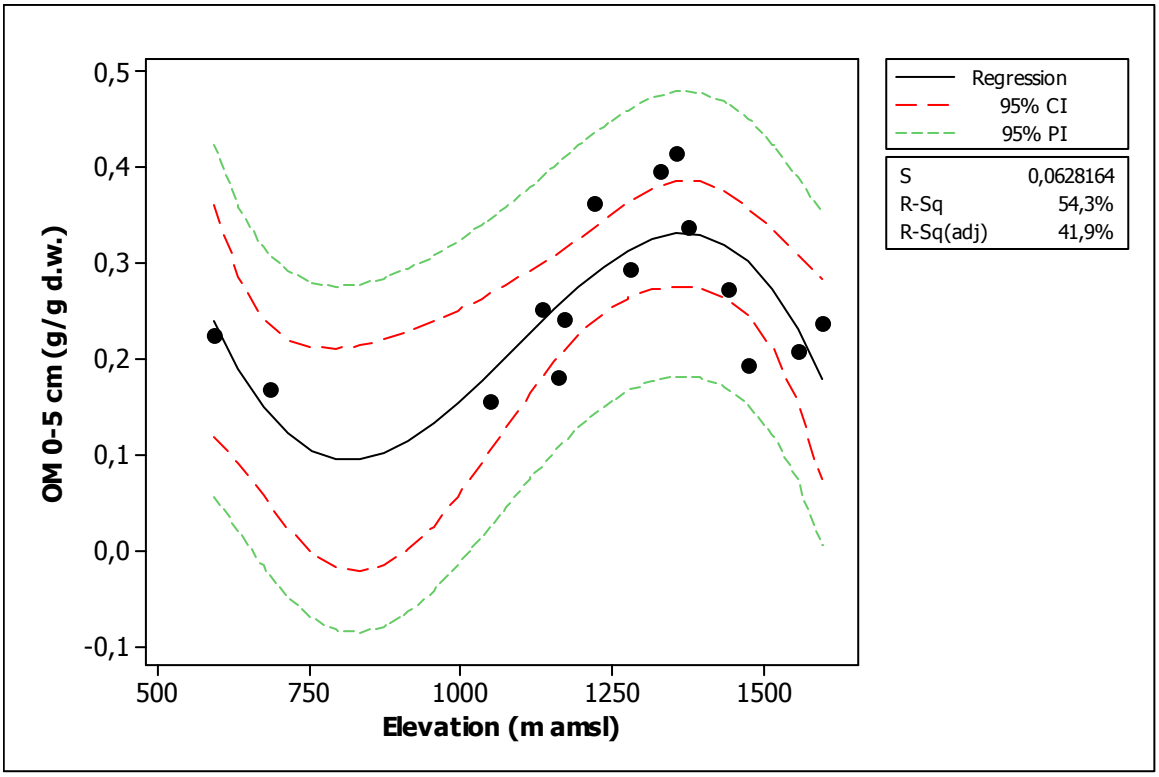


Figure 5-18: Polynomial (3rd order) regression between the concentration of O.M. in the 0-5 cm layer of the soil as dependent variable and elevation as predictor. Red lines represent 95% confidence interval, while green lines represent 95% prediction interval.

The polynomial relationship shown above was stable also in the deeper layers of the soil, although with a slightly decreasing determination coefficient. On this account, it was possible to build four

models predicting the concentration of organic matter (expressed as g O.M. on g dry weight) in the four soil layers. Given *e* as the variable elevation and *l* as one of the four layers, the equations were like:

$$\boldsymbol{OM}_l = a_l\boldsymbol{e}^3 + b_l\boldsymbol{e}^2 + c_l\boldsymbol{e} + k_l$$

With *a*, *b*, and *c* as coefficients and *k* as a constant.

In order to convert the values predicted by the models to a mass per area basis, it was necessary to take into account the bulk densities of the soil. It was always possible to find a significant and reliable linear combination of organic matter and bulk density per each layer as:

$$\boldsymbol{BD}_l = \alpha_l + \beta_l\mathbf{OM}_l$$

Thus, it was possible to express also the bulk density according to the equations for the organic matter as:

$$\boldsymbol{BD}_l = \alpha_l + \beta_l(a_l\boldsymbol{e}^3 + b_l\boldsymbol{e}^2 + c_l\boldsymbol{e} + k_l)$$

The final passage consisted of taking into account the volume of the soil cores, layer by layer, and report it to a square meter surface. Soil cores, being circular and with a diameter of 5 cm, had a surface of 19.625 cm^2 and a volume variable according to the length of the layer (either 5, 10 or 15 cm). To pass from the surface of the soil cores to a square meter basis, it is necessary to multiply for a factor of 509.55 (10000 cm^2 divided by the soil cores surface). Moreover, to pass from grams to kilograms, it is necessary a final division by 1000. Given *V* as the volume of soil cores layer by layer, the final equation to get the total organic matter as kg/m^2 in the soil from 0 to 40 cm depth is:

$$Total\ O.M.\left(\frac{kg}{m^2}\right) = \sum_{l=1}^{4} \frac{a_l\boldsymbol{e}^3 + b_l\boldsymbol{e}^2 + c_l\boldsymbol{e} + k_l \times [\alpha_l + \beta_l(a_l\boldsymbol{e}^3 + b_l\boldsymbol{e}^2 + c_l\boldsymbol{e} + k_l)] \times \boldsymbol{V}_l \times 509.55}{1000}$$

As already stated, this overall model allowed predicting with a considerable reliability the amount of organic matter, with a general trend to overestimation of maximum 30% of the value estimated by sampling. Given this model, it was possible to compute the amount of organic matter present in the main beech forests of the Italian Apennines using the covering areas of beech derived from the fourth level of the CORINE Land Cover project and a 1x1 km grid digital elevation model (DEM) obtained from the WorldClim dataset (Hijmans et al., 2005). The model has been plotted in a GIS software (QGIS) and the results offer the amount of organic matter expressed as $Mg \times 10^3$ per each cell, which has a size of 100 ha. (Fig. 5-19,20) Also the beech area present in the Alps is shown in the results, but the model has been calibrated for the Apennines and within an elevation range of 500-1700 m. Thus, the results coming from the Alps will not be taken into account.

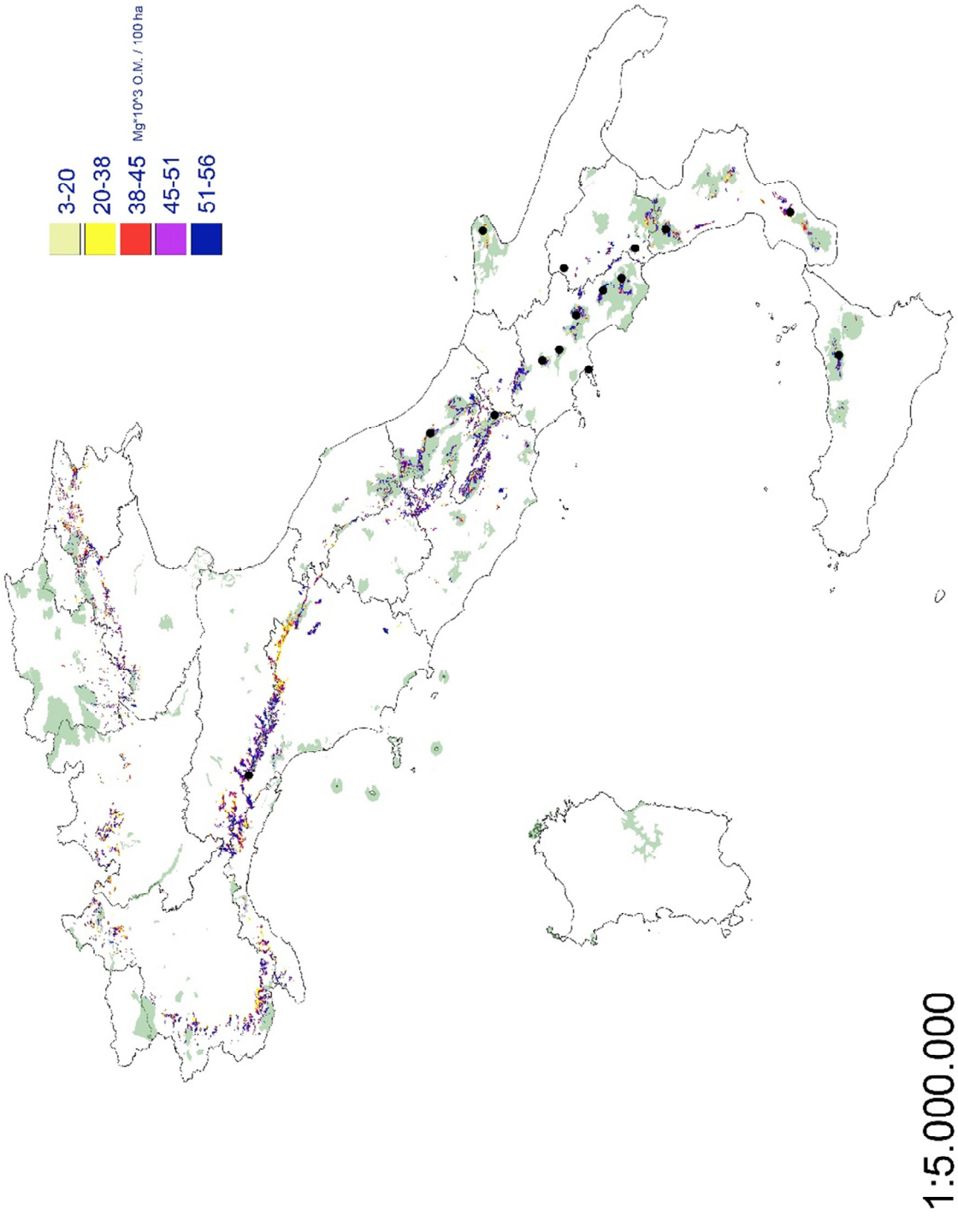


Figure 5-19: Map showing the estimated amount of O.M. as thousands of Mg in each grid of 1x1 km within the distribution of beech in Italy. The green areas represent National or Regional Parks or other protected areas. Black spots are the locations sampled in this study.

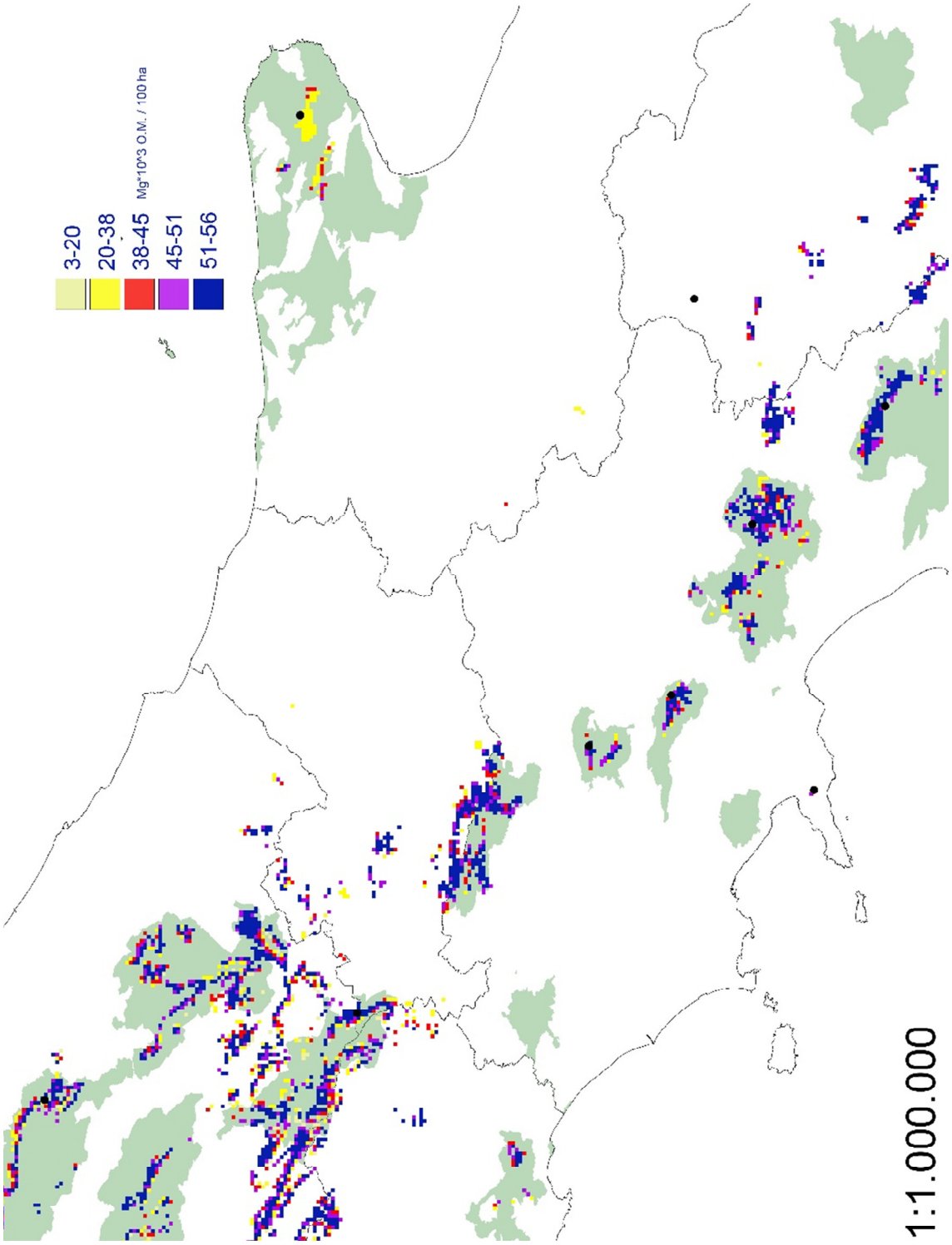


Figure 5-20: A close-up of the previous map showing the estimated amount of O.M. as thousands of Mg in each grid of 1x1 km within the distribution of beech in a detail of southern Italy (from parts of Abruzzo to parts of Basilicata). The green areas represent National or Regional Parks or other protected areas. Black spots are the locations sampled in this study.

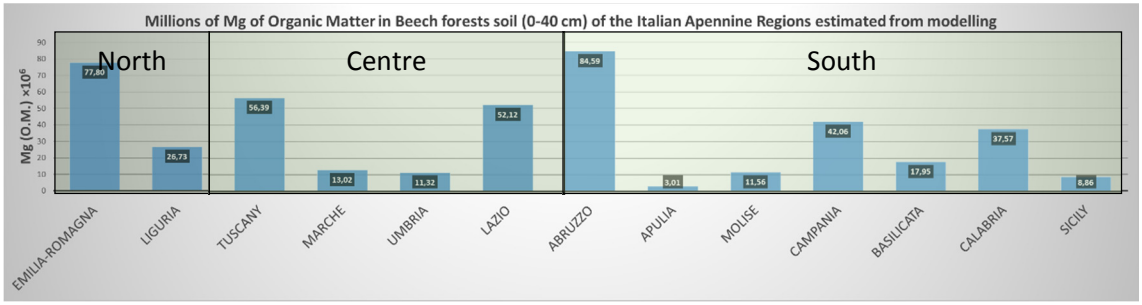


Figure 5-21: Amount of organic matter estimated from the model for beech forests at an elevation between 500 and 1700 m of the Italian Apennines administrative Regions.

The results point out the great importance of beech systems as pools of organic matter. The overall estimate for the amount of organic matter in beech forests between 500 and 1700 m altitude in the Italian Apennines is 443 million Mg. Dividing the regions geographically according to the Italian National Institute for Statistics (*Istituto Nazionale di Statistica, ISTAT*), the total amount is distributed 24% in the northern regions, 30% in the central regions and 46% in the southern regions. The largest pools are in Emilia-Romagna in the north, Toscana in the centre and Abruzzo in the south (Fig. 5-21). Region Abruzzo is historically considered part of southern Italy, but its geology and biogeography place it closer to the centre of the peninsula. Thus, excluding Abruzzo, in the south beech covering area is by far less extensive, thus the amount of O.M. stored in these forests is lower, although the sum of Campania and Calabria is as much as Emilia-Romagna.

Many of the beech forests of the Italian Apennines and some of those in heterotopic stands and/or in geographic areas non-directly along the Apennines, fall within protected natural areas. Of these, the most important are National Parks. Computing the amount of organic matter stored within the National Parks of the Italian Apennines is 25% of the total, with some parks (e.g Gran Sasso e Monti della Laga, Pollino, Maiella, Cilento e Vallo di Diano etc.) accounting more than the overall of regions like Puglia, Sicilia, Marche or Umbria (Fig. 5-22).

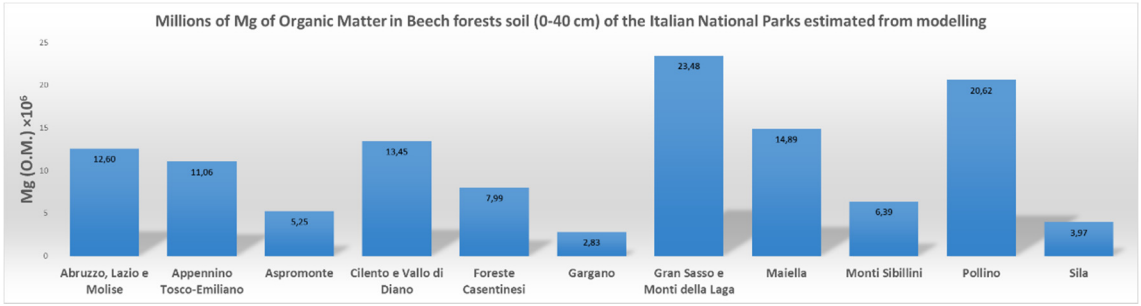


Figure 5-22: Amount of organic matter estimated from the model for beech forests at an elevation between 500 and 1700 m of the Italian National Parks.

5.4 Discussion

The results presented show a complex pattern of accumulation of organic matter in the leaf litter decomposition continuum and soil. Decomposition rates and annual litter production vary according to several variables and an unambiguous interpretation of all the results shown is not easy. Moreover, many other factors, some of which have not been considered in this study, concur to the accumulation of organic matter in the soil.

Among the factors that have been not directly investigated in this study there is, for instance, the impact of forest management and/or soil use. Although all locations have been selected in conditions that appeared to be natural and in old forests, forest management is surely still happening in many beech forests and had a major impact in the past. Forest management can, on the one hand, improve the potential C sequestration of forests while, on the other hand, disturbances can lead to unintended C losses (Jandl et al., 2007; Thuille et al., 2000). Forest age has also been shown to be a factor to consider in terms of quality of litter and, thus, decomposition rates and C turnover (Pregitzer and Euskirchen, 2004; Trap et al., 2013).

As for N and litter quality in general, Kooijman and Martinez-Hernandez (2009) found significant effects of parent material. Being the Italian Apennines a result of the Miocene orogeny from Mesozoic pelagic deposits (Rosenbaum, 2002), parent material was in most of the cases limestone and/or dolostone in our study. Thus the effects of parent material were not evident in the results and, surely, not the main cause of variability.

It has to be highlighted that not all of the organic matter is available for the biosphere as, by and large, the available forms of C and N are present in dissolved forms (Marschner and Kalbitz, 2003; Michalzik et al., 2001). Turnover times of the organic matter testify this diversity, as it increases with soil depth and it is generally 2–5 years for recognizable leaf litter, 5–10 years for root litter, 40–100+ years for low density humified material and >100 years for carbon associated with minerals (Gaudinski et al., 2000).

The results from this thesis showed a very high amount of organic matter in the soil for several important beech forested areas of Italy, many of them within or near National Parks of Italy. The presence of these protected areas have already been shown to positively affect the aboveground C stock when compared to the overall national territory (81.21 vs. 76.11 t/ha) (Marchetti et al., 2012). Compared to beech forests in Germany along a precipitation gradient (Meier and Leuschner, 2010), the presented results are comparable for the mineral part of the soil but not to the organic layers, where the described results are generally higher (although in the cited research mineral and organic layers are separated according to pedological features and organic matter content, thus not overlapping with all of the results).

This study presented an unprecedented estimation of cellulose, lignin and lignin-like substances in the soils until 40 cm depth. Although the method used gives only a proximate value, the data for

the soil give a very good insight of the dynamics of lignin which are considered as important components for the carbon cycle in soils. Lignin content of soil O.M. declines with decreasing size of the texture fractions, whereas its level of degradation increases concomitantly (Thevenot et al., 2010). A potential future implementation of this study could be a more detailed analysis of lignin related to the different texture classes within the soil, because the mechanisms of accumulation and potential stabilization of a part of lignins in soils, by interaction with the clay minerals, remain unclear (Thevenot et al., 2010).

As for the impact of ecological variables on organic matter and C cycle, some studies state that the role of warming could have been overestimated. Accordingly, with higher temperatures, there can be a reduction of soil carbon on the short term but, on the long term, the faster microbial respiration, that depletes carbon, is balanced by an increase of input to the soil for the higher net primary production and faster stabilization reactions of the organic matter in the soil compartment (Thornley and Cannell, 2001). Climate warming can, anyway, have other consequences. Meier and Leuschner (2010) concluded that climate warming along with a higher frequency and severity of summer droughts, as forecast for parts of Central Europe, will reduce the carbon stored in temperate forests dominated by beech. The recalcitrant and heavily humified organic matter (mostly derived by lignin or lignin like compounds) is usually stable within the soil, but it could become a source of "old C" with global warming, especially for the action of soil fauna, particularly Enchytraeid earthworms (Briones et al., 2010).

Being the Mediterranean region more highly susceptible of climate warming (Giorgi and Lionello, 2008), it is logic to foresee that, given the not so extensive range of elevations in the Italian Apennines, the beech would not be able to "climb" higher in the mountains to escape global warming. Besides, high amount of C stored in the soils could switch from sink to source (Belay-Tedla et al., 2009; Briones et al., 2010; Meier and Leuschner, 2010).

In north-south boreal forest soils, it was observed that higher temperatures and precipitation both concurred into a greater accumulation of C in the soils (Hilli et al., 2008) and this appears to be in contrast to some general remarks of this study. On the one hand, the study of Hilli et al. is not an excellent comparison to this study because it considered coniferous boreal forest above 60° latitude, which is already beyond the natural occurrence of beech forests. On the other hand, many findings in this study (i.e. the decrease of cellulose and increase of lignin along the decomposition continuum and in the soil and the trends of C/N) match the presented results. A positive correlation between rainfall and organic carbon accumulation have also been observed in a precipitation gradient study in Germany (Meier and Leuschner, 2010).

Dai and Huang (2006) proved that a correlation analysis indicates that surface soil O.M. concentration 886 data sets in China were generally negatively correlated with annual mean temperature (T) and positively correlated with annual mean precipitation (P) and elevation (H). These observations clearly match the findings in this thesis. A further investigation of these authors

suggested that multiple regression models with different combination of T, P and H could explain 41.5%–56.2% of the variability in surface SO.M. concentration for different geographical regions, even though the driving variables were different case by case.

The impact of elevation of forest ecosystems has already been proved important for conifers, as it can effect productivity, leaf chemistry, morphology and, thus, soil properties (Hultine and Marshall, 2000). A non-linear relationship between leaf morphological and physiological features has been proved also for *Quercus aquifolioides*, where specific leaf area, stomatal length and index increased with increasing altitude below 2800 m, but decreased with increasing altitude above 2800 m, whereas leaf nitrogen content per unit area and carbon isotope composition showed opposite change patterns (Li et al., 2006).

As for beech forests, elevation has been shown to be a very important factor in the Apennines and a significant quadratic relationship has been found with the length of the growing season, with optimum levels between 1300 and 1500 m (Bayat, 2011). These remarks suggest the validity of the modelling approach of this project.

For the beech data available for this thesis, there were not enough observations to assess a more or less simple multiple regression model, but a polynomial one was able to explain for the uppermost layer (0-5 cm) 42% of the variability using the sole elevation as a variable. The results evaluated from the model are in range with another study concerning forests in the Pacific Northwest region, USA (Homann et al., 2005). A statistical modelling approach in the mentioned study revealed the potential in terms of forecasting regional C dynamics and in land-management decisions related to C sequestration, and the results from this thesis could be adopted in the same direction.

As for Italy, in 2005, a large-scale Italian project by the State Forestry Corps (*Corpo Forestale dello Stato*), the Research Unit for Forestry Monitoring and Planning (*Unità di ricerca per il Monitoraggio e la Pianificazione Forestale*) and the Ministry of Agriculture, Food and Forestry (*Ministero delle Politiche agricole alimentari e forestali*) published a National Inventory of Forests and Forests Carbon Sinks (*Inventario Nazionale delle Foreste e dei Serbatoi Forestali di Carbonio, INFC*) (http://www.sian.it/inventarioforestale/index.do). The results of this inventory include the estimate of the carbon content by means of physical and chemical analysis conducted in the laboratory on over 18,600 samples of litter and soil for all sort of forests and woods in Italy, including a subsample of beech forests. Organic carbon has been estimated in the litter and soil. In the soil, an organic layer and mineral layers were separated, subdividing the latter into a surface layer, 10 cm thick, and a deeper layer, 20 cm deep. The results from the National Inventory of Forests and Forests Carbon Sinks for the overall organic C in litter and soil of the different regions can be compared with those predicted by the model developed in this work by simply dividing the amount of organic matter by a factor of 2, in order to estimate the organic C. It can be seen that, neat of the different methodologies, sampling size and biases, the amount of C predicted by the polynomial model follows the same trend of the INFC, although there is a clear leaning to overestimation. Nevertheless, the

relationship between the two values is highly significant (p<0.001) and the R^2adj. very high (0.639) (Fig. 5-23).

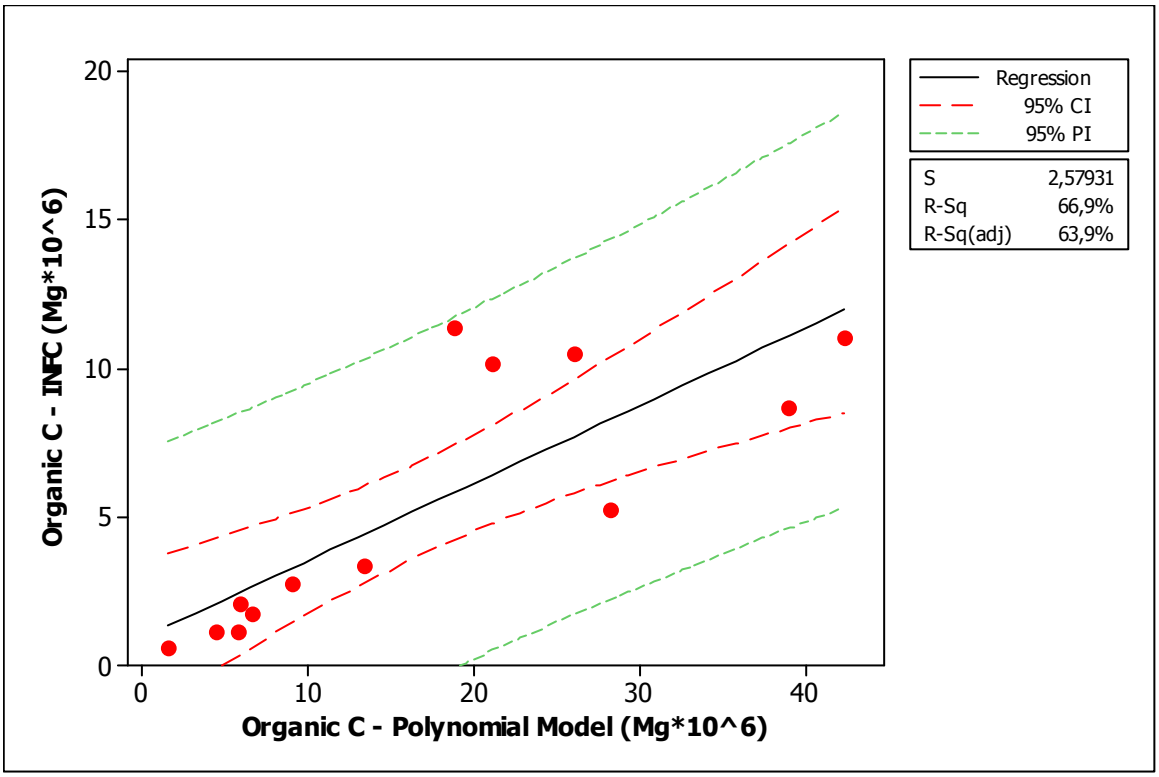


Figure 5-23: Relationship between the values of C estimated by the INFC and those obtained by converting the values of O.M. gained from the polynomial model.

The results from the polynomial model adopted in this thesis are biased by overestimation, especially for the deeper layers. Moreover, given the complexity of the model, there is a risk of over fitting. Yet, the good correspondence in relative trends between the values from the model and those from the INFC suggest that the principle of the relationship between organic matter and altitude is robust. Future developments of this model are in plan, after an expansion of the dataset and a series of field verification in order to perfect and validate the model.

The overall results from this last section of this work, although biased by a low sampling number, by a sizeable error and by a non-pedological characterization of the soils, allow a good and detailed estimation of the millions of Mg soil organic matter. In principle, the model can be used as a first sound estimation of organic matter in every Italian beech forest between 500 and 1600 m. Besides, the large amount of data and material collected can be considered the basis for several future investigations into different directions concerning quality and quantity of organic matter in Italian beech forests. This can be especially important for the deeper layers of the soil, where the scarce available data suggest that the relationship between organic matter biochemical quality and position within the soil profile can be complex and thus deserves further attention (Rovira and Vallejo, 2007).

6 Conclusions

The main findings and conclusions of this Ph.D. can be summarized as follows:

- A clear and unambiguous pattern of decomposition in the beech forests of Europe cannot be assessed but, on average, the decomposition on the Italian Apennines appear to be slower than most of the cases in Middle Europe.
- In terms of loss of weight, on the field plant quality is a driving factor for the first phase of the decomposition (200 days), while the different locations increase its relevance in the later stages (200 to 680 days).
- Under laboratory condition, where the focus is more only on the very early stages of decomposition, a higher impact of the soil type at the beginning of the experiment (first 30 days) was showed, whereas plant material becomes significant later on (60 to 90 days) and multifarious interaction between plant material, soil and climatic conditions are triggered, suggesting a complex microbial response to the different factors.
- The comparison between the results produced by the decomposition experiment on the field and the ones gained under laboratory condition put forward that, even though on the field the leaves decaying in the forest of Pradaccio (colder climate) have a smaller decomposition constant, when this soil and its microbial communities undergo favourable constant conditions of temperature and humidity the decomposition on the soil of Pradaccio is faster, at least for the early stages of decomposition.
- The different behaviour of the two microbial communities in the soils suggest that, for a colder site, higher temperatures are a favourable condition, whereas for a hotter site they slow down decomposition because, in terms of adaptation of the soil biota, higher temperatures are related to aridity. Likely, the soil biota in Laceno is adapted to good functionality for longer periods during spring, autumn and parts of winter since they are hindered by the semiarid and warm summer. On the contrary, in Pradaccio, where the temperatures are much more rigid, the snow cover is far more abundant and the summer drought is not so stressed, the microbial communities are expected to be more active in late spring and summer.
- Most of the enzymes sampled in the on-the-field experiment (except dehydrogenase) show a highly significant negative correlation with the mass loss. This implies that a careful analysis of the different trends can give a good insight at what is happening and which compartment of the microbial community is likely involved.
- This study confirms that the degradation of lignin and cellulose is very slow at the beginning but, after some time, the activity of cellulase and xylanase greatly increase.

At the same time, the activity of laccase and peroxidase slowly increase and they reach their maximum activity once the cellulase and xylanase slow down. Clearly, the decomposition of holocellulose starts only when peroxidase and laccase begin the aggression of lignin, exposing the holocellulose. Once the holocellulose is decreasing, the activity of laccase and peroxidase strongly increase, as lignin starts to become the most abundant plant tissue remaining.

- The shifting between laccase being higher in Laceno and peroxidase higher in Pradaccio are connected to the faster decomposition happening in Laceno and to the higher availability of Mn in Pradaccio. The correlation between peroxidase and Mn can be appraised by a linear regression of the activity of peroxidase and the increase of Mn during decomposition (R^2adj.=0.145, p=0.001).
- On a general line, even though both the soil and litter quality appear to be higher in Pradaccio, on the field the decomposition is higher in Laceno, while in laboratory conditions decomposition proceeds faster for both plant and soil from Pradaccio.
- This study confirms the importance of Mn in the decomposition process which, according to the evidence given in this thesis, has a major role not only in the later stages of decomposition but also in the early ones. The dynamics of Mn availability is unknown, but may be related to its initial concentration and this thesis confirms this trend which has been observed few times. In fact, it has been showed an increase of Mn more noticeable in those leaves (Laceno) which had a lower initial Mn concentration. This suggests that, even from the early stage of decomposition, fungi colonizing the leaves recruit Mn from the environment (soil or more decomposed material) with a higher rate when this nutrient is limited in the litter. This evidence strengthens our suggestion that Mn is highly transported in the plant material when lignin starts its decomposition, while it becomes a source once lignin decay slows down because of the limiting effect of N in the higher decomposed layers.
- The lower concentration of Mn from our study compared to other studies could also contribute to explain the differences between decomposition in mountain ecosystems like the Italian Apennine and those in Middle Europe, which generally prove to be faster. A correlation with the higher precipitation of mountain ecosystems and the lower concentration of Mn could be, instead, hypothesized.
- The results from the seasonal analyses showed a sensible reduction of organic matter (and thus C and N) along the decomposition continuum. The relationship between the concentration of C and cellulose and N with lignin suggest the trends of decomposability/recalcitrance of this compounds along the decomposition continuum and along depth.

- The content of organic matter is shown to be much higher in Pradaccio than Laceno in the uppermost layer, but higher in Laceno in the deeper layers. Litter quality is higher in Pradaccio, and also soil quality appears to be higher, as shown in the litterbags laboratory experiment. Thus, even tough on the field the decomposition is faster in Laceno, there is a higher amount of organic matter in the soil. The explanation could be derived to the higher annual litter input of Laceno, due to the longer vegetative season which allows to grow larger leaves and in greater quantity.
- Microbial activities were even higher deeper in the soil in comparison to the abundance of organic matter. For some very specific C sources, as lignin-like substances, the activity of laccase and/or peroxidase increased strongly in deeper layers of the soil, suggesting the presence of differentiated and specialised fungal communities.
- The patterns of changes in litter quality reflected what happens in the litterbags, but some increases (e.g. the increase of N and Fe) and much broader than what happens in the litter bags. The phenomenon has been shown to be more extensive for the summer season in both locations, although with the differences in absolute and relative concentration which was already seen in the litterbags. The differences in the values could be easily explained by the filter effect of the litterbags which did not allow the access to all of the soil fauna. The activity of soil fauna could be also an explanation for the increase of organic matter and the changes of C:N ratio during summer.
- The principal component analysis, for both litter and soil, always confirmed the greater diversity of the summer season compared to the spring/autumn. Biplots clarified that, for litter, summer showed a change in all the parameters of the litter quality while autumn/spring had more diversity for the enzyme activities. In contrast, in the soil, a set of enzyme activities contributed to explain the greater diversity of the summer season, even deeper in the soil. Differences could be appreciated also between the locations, with a shifting of some enzymes activities between Laceno and Pradaccio.
- Both locations clearly showed during summer an increase in nutrient contents in the decomposition continuum of the litter that could be explained by the increase in soil fauna activities. The activities of earthworms contributes in retranslocating organic matter in available forms from the upper layers to the deeper layers, enhancing the microbial enzyme activities even in deeper layers of the soil. In the litter (and partly in the soil), enzyme activities appear to be generally higher in Laceno during autumn and spring, while for Pradaccio the peak is generally more shifted to the summer period, although some activities were relevant also in autumn. This evidence can be supported by the already mentioned climatic differences and the litterbags experiment, and the drought summer periods (more evident in Laceno) appear to be the greater factor

slowing down the microbial activity. For Pradaccio, the limiting factor is likely the colder climate and the snow cover, with the whole soil phenomena more concentrated between May-October, with summers as a particularly positive time of the year.

- The results from the north-south Apennines transect of this thesis showed a very high amount of organic matter in the soil for several important beech forested areas of Italy, many of them within or near National Parks of Italy along with an unprecedented estimation of cellulose, lignin and lignin-like substances in the soils until 40 cm depth.
- For the beech data available for this thesis, there were not enough observations to assess a more or less simple multiple regression model, but a polynomial one was able to explain for the uppermost layer (0-5 cm) 42% of the variability using the sole elevation as independent variable.
- The results from the National Inventory of Forests and Forests Carbon Sinks for the overall organic C in litter and soil of the different regions can be compared with those predicted by the model developed in this thesis. Neat of the different methodologies, sampling size and biases, the amount of C predicted by the polynomial model follows the same trend of the INFC, although there is a clear leaning to overestimation. Nevertheless, the relationship between the two values is highly significant (p<0.001) and the R^2adj. very high (0.639), suggesting that the principle of the relationship between organic matter and altitude is robust.

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