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Soil Organic Matter and Carbon Sequestration in Forest Stands on Mount Vesuvius

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Minds are like parachutes:
they only function when they're open
Thomas Dewar

to Raffaele Pio
… my nephew

Abstract

Soil organic matter in forest ecosystems represent an important C pool. Litter fall is the largest source of organic material and nutrients for the organic and mineral layers in forest ecosystems. The chemical composition of plant litter has a large influence on soil microbial communities and is one of the main factors affecting litter decay rates and the dynamics of SOM. Introduction of alien species, in forest ecosystems, may have a potential allelopathic effect on other tree species and allelopathic chemical compounds produced by alien tree species might inhibit soil microbial activity with consequent effects on storage rates of SOM and ecosystem nutrient cycling. Until know little attention has been paid to this topic that is worthy of investigation because alien species are largely used for afforestation of poor soils or easily invade bare soils.

Forest stands at Mount Vesuvius are a convenient study area to investigate C sequestration in soil. Chronosequences of tree plantation occur on volcanic substrate of know age; moreover different species of trees have been used for afforestation including the alien invasive species Black locust (*Robinia pseudoacacia L.*). Thus it is possible to study carbon sequestration in the soil as related to the age of the stand and to type of tree cover.

In the two coeval (36 yrs old) stands on lapillus of the last eruption of Vesuvius, with a different tree cover (Black locust and Corsican pine, *Pinus nigra* Arn. var. *corsicana*), litter fall (years 2006–2008) is higher in the Corsican pine than in the Black locust stand ($P < 0.05$). Total Litter Fall over Basal area (TLF/BA) is 90.7 Kg/m^2 for Black locust vs 210.2 Kg/m^2 for Corsican pine and the higher litter fall amount of Corsican pine corresponds to the higher biomass. In the organic soil layers amounts of organic C are higher ($P < 0.05$) in Corsican pine as compared to Black locust stand (g/m^2 = 2701.9 vs 1636.4), while in the mineral layers organic C amounts are lightly higher (but not statistically different) in Black locust than in

Corsican pine soil (136 vs 116 g/m^2). ^{13}C CPMAS NMR data show that the aromaticity degree of humus is higher for Black locust than for Corsican pine (% Ar = 9.54 vs 7.08).

In the Stone pine cronosequence, litter fall (2006–2008) increases gradually from the younger stand (36 yrs old) to the oldest one (96 yrs old) and is positively related to tree basal area (BA); Total Litter Fall over Basal Area (TLF/BA) also increases with stand age (123 to 145 Kg/m^2). Moreover chemical analyses of organic and mineral soil layers clearly indicate the accumulation of C with stand age (g/m^2= 1053.9 for 36 yrs old stand vs 2523.5 for 66 yrs old one and vs 3065.2 for 96 yrs old one).

The results dealing with the black locust stand allow to conclude that Black locust litter: i) inhibit soil microorganisms activity, ii) produces a recalcitrant residue, rich of aromatic compounds, including 4–hydroxyacetophenon (indentified by ^{1}H NMR), known for its allelopathic potential. This may lead to high accumulation of organic matter in the soil and thus to high C sequestration.

The comparison of all pine stands shows that the trend of litter fall and C sequestration, increases from the younger to the older stand.

Key words: *C sequestration, coeval stands of Corsican pine and Black locust, Stone pine cronosequence, litter allelopathic effect, 4–hydroxyacetophenon, soil microbial activity.*

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1. INTRODUCTION

Organic matter plays a key role in determining most soil properties. Soil organic matter (SOM) consists of a heterogeneous mixture of interacting mainly polymer compounds (Sposito, 1989) and originates from carbon (C) fixed by plants and delivered to the soil in the form of leaf and wood litter, dead roots, and root exudates; soil organic carbon (SOC) also includes animal and microbial residues in all stages of decomposition. Many organic compounds are intimately associated with soil minerals.

SOM is the largest carbon reservoir that is in exchange with atmospheric CO_2, and is thus important as a source and sink not only of CO_2 as a carbon source for plants but also for the greenhouse gas over time scales of human concern (Schimel, 1995). The turnover time of different SOC compounds vary due to climate changes (e.g. Trumbore and Torn, 2003; Liu et al., 2004; Akselsson et al., 2005) and to the complex interactions between biological, chemical and physical processes in soil and ranges from hours, for highly decomposable carbohydrates such as simple sugars exuded by roots, to millennia, for C associated with noncrystalline minerals (allophane, imogolite and ferrihydrite) (Torn et al., 1997; Trumbore, 1997; Stevenson and Cole, 1999) and for C to humus in undisturbed forest soils (Wardle et al., 1997). Numerous cronosequence studies support the idea that, in the absence of disturbance, amounts of organic matter continue to increase in soils over centuries or even millennia (Wardle et al., 1997; Jenny, 1980).

Forest ecosystems represent an important C storage pool since they are estimated to contain 82–86 % of the global terrestrial C (Sedjo, 1992; Dixon et al., 1994; Houghton et al., 2001), which suggests that management and disturbances of forest may have a profound effect on the C cycling on a global scale. In terrestrial ecosystems the amount of carbon in soil is usually larger than that in the vegetation. It is therefore important to understand the role of soil carbon in the carbon balance in the terrestrial ecosystem and the global carbon cycle.

Forest ecosystems in different climate zones and biomes may have SOM with different concentrations of nutrients and thus store different amounts of nutrients in the accumulated SOM (Berg and Laskowski, 2006), and their availability is related to the decay dynamics of the organic matter in the soil (Berg and McClaugherty, 2008). The organic matter decay is carried out by the soil microbial community, which with its rich diversity, regulates the fluxes of C and N on both a local and a global scale.

Cronosequence are generally soils under similar conditions of vegetation, topography, and climate (Harden, 1982), and are used in global change research to predict long term changes in the carbon pools and fluxes ecosystems (Schöning and Kögel–Knabner, 2002).

Carbon inventories in SOM have shown increases and decreases in amount of C along a cronosequence developed on lava and ash deposits of different age in the Hawaiian island chain (Trumbore and Torn, 2003). The increase and decline in amount of C has been linked to changes in the amount and type of soil minerals that can stabilize SOM (Oades, 1988; Torn et al., 1997). The rate of C inputs to SOM are determined by the net productivity of vegetation, thus, for soils where organic matter decomposition patterns are similar, the soils with more productive vegetation will have higher stores of organic C (Trumbore and Torn, 2003). On the other hand, with similar amounts of litter fall among stands of different tree species, the decomposition pattern may determine the amount of carbon e.g. in a humus layer (Vesterdal and Raulund–Rasmussen, 1998; B.Berg, personal communication).

Plant species reinforce existing patterns of nutrient availability (Wedin and Tilman, 1990; Hobbie, 1992), thus controlling, also, C storage, nutrient cycling, as well as physical and chemical characteristics of soil and soil microbial activity, tightly related to decay process. In general, leaf litter with a high nitrogen (N) concentration is favored by both bacteria and fungi and will decompose rapidly (Melillo et al., 1982). Invasive species often have higher concentrations of leaf nitrogen (Vitousek et al., 1987; Vitousek and Walker, 1989; Witkowski, 1991; Baruch and Goldstein, 1999; Nagel and Griffin, 2001) and, consequently, exotic species introduces in a new area, are expected to decompose more rapidly and release more nitrogen to soil than native species; this can have an effect on soil microbes and thus a strong influence on nutrient cycling (Vitousek, 1990; Ehrenfeld, 2001).

2. FOREST ECOSYSTEMS

An ecosystem is a natural unit consisting of biotic factors (plants, animals and micro–organisms) and of non–living physical (abiotic) factors of the environment (Christopherson, 1996).

Forest ecosystems represent one of the most important units of the Earth's biosphere, with high density of trees, other vegetation (bushes, herbaceous species, etc.), litter and soil, each having its own temporal dynamics, carbon storage patterns, and carbon release rates to the atmosphere. Forests can be found in all regions capable of sustaining tree growth, at altitudes up to the tree line, except where natural fire frequency or other disturbance is too high, or where the environment has been altered by human activity.

The concept “forest” can be classified in different ways and to different degrees of specificity. One such way is in terms of the "biome" in which they exist, combined with leaf longevity of the dominant species (whether they are evergreen or deciduous). Another distinction is whether the forest is composed predominantly of broadleaf trees, coniferous, or mixed.

Trees live and grow over periods ranging from decades to hundreds of years. When trees die, the carbon sequestered over the years in the live biomass is transferred to litter and soil, and is released to the atmosphere, or incorporated in the soil or transformed into forest products.

Forests create highly heterogeneous environments: an infinite number of possible spatial arrangements of trees of varying size and species makes for highly intricate and diverse micro–environments in which environmental variables (temperature, solar radiation, relative humidity, and wind speed) can vary greatly over large and small distances. Normally an important proportion of a forest ecosystem's carbon pool is underground, where soil structure, water quality and quantity, and levels of soil nutrients can vary greatly.

Thus, forest ecosystems represent an important C pool since they contain 82–86% of the global terrestrial C (Sedjo, 1992; Dixon et al., 1994; Houghton et al., 2001). The amount of C stored in forest soil is about 1,24 Pg (1 petagram = 10^{15} gram) (Houghton, 2008). The amount of the global soil N, estimated until 1 m depth, is 133–140 Pg (Batjes, 1996; Tian et al., 2006). Soil C and N stocks and dynamics are influenced by vegetation, fire and anthropogenic factors, causing elevated atmospheric CO_2 concentrations and depositions or losses of NH_4^+ and NOx (Dixon and Turner, 1991; Dise and Wright, 1995; Batjes, 1996; Townsend et al., 1996; Harrison et al., 2000; Persson et al., 2000).

Stand age and tree species have an influence on amount of soil organic matter. A decrease in C assimilation efficiency with stand age has been found (Bauhus et al., 1998). Schulze et al. (2000), suggested that natural forests can represent a steady C accumulation potential up to the over–mature phase, considering all possible influencing factors (as fire – causing a C accumulated decrease – or anthropogenic influences). Berg and McClaugherty (2008) reviewed that therewere no real differences in humus accumulation rates between young and old stands. Further, considering the growth rates shown by Wardle et al. (1997) and compared to that of Staaf and Berg (1977) any form of steady state appears to be very distant in time.

2.1 Litter fall

Litter fall is the largest source of organic material and nutrients for the humus layer in forest ecosystems. The quality and quantity of litter fall influences the nature of the microbial community, including its size, composition, function and physiological properties. The composition of the microbial community may, in turn, influence the course of decomposition and the chemical changes in the litter during decomposition. Transferring energy bound to the vegetation to the soils, litter fall is a dominant link in the biogeochemical cycling of matter.

Plants shed not only foliar litter, but, with trees as an example, twigs, branches, bark, roots, flowers, and occasionally cones. Structures such as cones and other fruiting bodies are often quantitatively important and may sometimes exceed foliar litter as the largest component. Several litter types are not “recently dead” but are recognized as litter after they have been shed and started to decompose and their chemical composition has begun to change. This applies, for example, to twigs, branches attached to the trees and boles that remain standing after their death and often start decomposing before they fall to the ground and are recognized as litter. Roots die and are “shed” differentially based on their size and function, and dead roots may remain attached to their parent tree for extended periods.

Quantity and quality of litterfall varies with tree species, geographical position, stand age and density (Albrektson, 1988; Berg et al., 1995; Liu et al., 2004). Investigations of forest litter fall at a variety of geographical scales is an essential strategy to study the relationships to environmental factors (Matthews, 1997). At a regional scale, precipitation and temperature are the most important climatic factors controlling ecological processes. Some large compilations have been published on litter fall. Liu et al. (2004) calculated litter fall for Eurasia and found separate patterns among the climatic zones; e.g. in the boreal zone the litter fall rate was higher in coniferous as compared to deciduous forests. Meentemeyer et al.

(1982) used data sets with nearly global coverage and related litter fall with e.g. actual evapotranspiration (AET), and other climatic variables. In a regional study for Fennoscandia Berg and Meentemeyer (2001) related foliar litter fall to AET. However, the impacts of temperature and precipitation separately may be better analysed by using them as single factors. Litter fall, due to differences in physiology and ecology between tree species, in some kinds of forests is possibly more related to temperature, and in other ones to precipitation. Among regions temperature and precipitation may shift as the most regulating climatic factor. Different patterns of foliar litter fall among species can be distinguished in the boreal and temperate zone. There is not only a difference between the deciduous and coniferous trees as groups but also among species within each group (Berg and Laskowski, 2006).

In the northern hemisphere the evergreen conifers, due to their evergreen nature, have a higher litter production compared to deciduous broadleaf forests, although a few exceptions exist (Bray and Gorham, 1964). Berg and Laskowski (2006) summarized some litter fall patterns: not all needle litter species on a shoot are shed at the same time but e.g. for spruce (*Picea*) single needles die and stay attached dead for several months before they finally fall, while, e.g. for pine (*Pinus*) and among deciduous trees, there is normally a heavy litter fall during a short period in the autumn when the trees shed all or a main part of their foliage, e.g. *Pinus* all needles on the oldest shoot.

Within a groups of stands on soils of similar richness and under climatically similar conditions, annual leaf and needle fall may be related to stand properties, such as stand age, basal area, or canopy cover, properties related to stand development. Litter fall for such a developing single stand may be modelled, e.g. over a stand age (e.g. Berg et al., 1995; Berg and Laskowski, 2006). Information needed encompasses, i) time for canopy closure (canopies do not expand further), ii) stand age, iii) quantitative litter fall. Such information is ideally collected in a cronosequence including a mature stand. Berg and Laskowski (2006) have presented two simple models of litter fall over a stand age:

- *Linear model*, litter fall increases linearly from a stand age of one year up until canopy closure, after which the litter fall may be considered constant.
- *Logistic model*, litter fall increases initially at an exponential rate until about a maximum canopy cover, when the increase rate slows down approaching an asymptotic level, and litter fall becomes about constant.

A common method to sample litter fall is to use circular litter traps, often of 0.25 m^2, mounted at a height of c. 1 m above the ground, with the collector bag being a loosely hanging net on a metal or wooden frame. Such traps were recommended already in the International Biological Programme (IBP Newbould, 1967). A common number is between 10 and 20 replicate traps per stand, with plot sizes ranging between 2500 m^2 and a hectare.

Litter traps intended to collect foliar litter can be placed randomly over the plot. The net mesh size can vary:

- for needles of spruce or larch the mesh size should preferably be less than ca 0.20 x 0.20 mm
- for beech and oak leaves (broadleaf in general), a mesh size could be of 1 cm.

Other litter components such as twigs, branches and most fruiting bodies like cones or acorns have no even distribution over the ground but fall directly under the canopy. Thus, traps for these components could ideally be placed to reflect the canopy projections in the ground. This means either randomly, depending on canopy density, or directly under the canopies. Of course, a high enough number of traps randomly placed and reflecting also the canopy distribution can be used. In a year sampling periods have frequency of every one to three weeks for foliar litter from conifers or evergreen. In contrast, for those deciduous species that shed the main part of their foliar litter during a shorter period only, samplings may be carried out during a more limited period. The sampling frequency is important from both the point of view of quality and quantity since, e.g., nutrients and soluble compounds may be leached out by rain and a wet litter may start decomposing and thus lose mass.

2.2 Soil organic matter and its accumulation

The term "Soil Organic Matter" (SOM) is generally used to represent the organic constituents in the soil, including undecayed plant and animal tissues, their partial decomposition products, and the soil biomass. Forms and classification of soil organic matter have been described by Tate (1987) and Theng et al. (1989). SOM consists of a variety of components and may be divided into the following fractions:

- *aboveground organic matter*, comprises plant residues and animal residues on top of mineral layer;
- *belowground* or *active organic matter*, consists of living soil fauna and microflora, partially decomposed plant and animal residues, and
- *resistant* or *stable organic matter*, also referred to as *humus,* unidentifiable components.

Even this apparently simple distinction, however, is not as clear cut as it might appear. One of the major problems is the lack of precise definitions for unambiguosly specifying the various fractions. Unfortunately, the terminology is not used in a consistent manner.

The term *humus* is used by some soil scientists synonymously with *soil organic matter*, e.g. for Waksman et al. (1928), humus or soil organic matter is the sum total of the stable organic substances in soil not including undecayed animal and plant tissues, partially decomposed material and the live roots and soil microbial biomass.

In the CENTURY model, Parton et al. (1987) partitioned SOM into three major components:

1. a small (10–40%) 'passive' pool with stabilized or physically protected material that can have a mean residence time of thousands of years,
2. a large (60–85%) 'intermediate' pool with turnover times that range from less than 10 to more than 100 years,
3. a smaller (~ 5%), highly labile 'active' pool consisting of microbial biomass and easily decomposable compounds.

CENTURY model attributes roughly 80% of heterotrophic soil respiration to decomposition of active SOM, with the remaining 20% arising from the intermediate pool, and turnover of all pools is controlled by the same exponential temperature function (Schimel et al., 1994).

Soils contain thousands of different low to high molecular weight compounds that vary dramatically in their resistance to decomposition, with fractions of SOM that vary in turnover time from days up to thousands of years (Kononova, 1975; Schlesinger, 1977; Van Veen and Paul, 1981). The most labile pools comprise only a small fraction of the total, but because they turn over so rapidly, day to day soil CO_2 efflux is dominated by their dynamics (Schimel et al., 1994), while more recalcitrant pools dominate the SOM inventory (Trumbore, 1993).

SOM is any material produced originally by living organisms (plant or animal) that is returned to the soil and goes through the decomposition process. At any given time, it consists of a range of materials from the intact original tissues of plants and animals to the substantially decomposed mixture of materials known as humus. Otherwise, after the initial decomposition has taken place the continued decomposition of the litter changes the chemical composition and structure as the litter approaches humus.

Although soil organic matter can be partitioned conveniently into different fractions, these do not represent static end products. Instead, the amounts reflect a dynamic equilibrium. The total amount and partitioning of organic matter in the soil is influenced by soil properties and by the quantity of annual inputs of plant and animal residues to the ecosystem. For example, in a given soil ecosystem, the rate and pattern of decomposition and accumulation of soil organic matter is determined by such soil properties as texture, pH, temperature, moisture, aeration, clay mineralogy and soil microbiological activities. A complication is that SOM in its turn influences or modifies many of these same soil properties. Organic matter existing on the soil surface as raw plant residues helps protect the soil from the effect of rainfall, wind and sun. Removal, incorporation or burning of residues exposes the soil to negative climatic impacts, and removal or burning deprives the soil organisms of their primary energy source.

Litterfall from trees is a main source for soil organic matter (SOM) in forest ecosystems. Plant residues contain carbon (C), oxygen (O), hydrogen (H) and small amounts of sulphur (S), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) plus further nutrients. Although present in small amounts compared to carbon, these nutrients are very important from the viewpoint of soil fertility management. The C:N ratio is sometimes used to indicate the type of material hard woody materials with a high C:N ratio being more resistent than soft leafy materials with a low C:N ratio, even if wood stems may be decomposed within a year (Mark Harmon personal comment).

SOM may be seen as a sink for C (Berg et al., 1995) and the accumulation rate in such a sink may depend on the chemical composition of newly shed litter (Berg et al., 1996). The chemical composition of plant litter has a large influence on the soil microbial communities and is one of the main factors affecting litter decay rates and the dynamics of SOM.

Soil organic matter in forest ecosystems has a key role for both nutrient availability and storage of different nutrients in the soil. Thus, intensive forestry practice, impacts of acid rain, high deposition of N or heavy metals may influence quantity and quality of organic matter in the soil.

Long–term accumulation of humus or soil organic matter (SOM) and thus of carbon (C) takes place in undisturbed forest systems (Ovington, 1959) which has been confirmed in cronosequence studies, with ages ranging over millennia (e.g. Wardle et al., 1997). According to the influence of stand age and tree species on soil organic matter quality, Bauhus et al. (1998) found a decrease in C assimilation efficiency with stand age as well as less microbial biomass beneath conifers compared to deciduous species. Furthermore, ^{14}C dating has indicated that SOM can be 10,000 years old (Paul, 1984; Stevenson, 1994), with the upper limit still not determined.

Soil carbon turnover and storage are controlled in large part by climate (Jenny, 1980), but the heterogeneous nature of SOM complicates analyses of the relationship between decomposition and climate. Simply knowing the amount of carbon stored as SOM does not give much insight into the importance of the soil C reservoir for influencing atmospheric CO_2. For example, if most C in soils is extremely stable, the time for the soil C reservoir to respond to a change in climate or C inputs could be quite long. The time scale of interest for studying how soils may influence atmospheric CO_2 is generally less than a century or more than a million years. The steady increase in radiatively active gases in the atmosphere is likely to raise global temperatures over the coming century (IPCC, 1992).

Hence it is evident that the rate of buildup of a soil organic matter (SOM) layer depends not only on the amount and quality of the litter formed but also on rate and pattern of its decomposition.

2.2.1 Soil microbial communities

Aboveground (plant community) and belowground (soil community) components of terrestrial ecosystems are closely related (Bardgett, 2005). In the form of plant litter and root exudates, plants supply a carbon and energy source and other nutrients to the soil decomposer community, and in turn, the soil biota, particularly its microbiota, decomposes soil organic matter, stabilizes soil structure and, through its essential role in the cycling of elements, releases nutrients for plant growth (Vitousek, 1990; Ehrenfeld et al., 2001; Porazinska et al., 2003).

In terrestrial ecosystems abiotic factors are well established as the dominant factors determining the vegetation patterns, but, recently, also biotic interactions in the soil have been reported as having major influences on the composition of plant communities (Hooper et al., 2000; Wardle, 2005).

It is important to be aware of the complex patterns of biodiversity in terrestrial ecosystems and, above all, the above– and belowground relationship that influence ecosystem function. Biodiversity in soil is extremely high, particularly at the microbial scale (Torsvik et al., 1994), and further, there is a large number of trophically equivalent organisms: most microbial species in soil must be functionally redundant (Bardgett, 2005; O'Donnell et al., 2005). For this reason, measurements of functional diversity in soil communities are likely to provide information more relevant to the functioning of soil than species diversity (Zak et al., 1994). Soil functional diversity depends on numerous metabolic reactions and interactions of biota, and – as an example – it is certainly unrealistic to assume that a simple relationship might exist between a set of enzyme activities and soil functional diversity (Nannipieri et al., 2002).

It has been reported that both plant species and plant diversity are major factors affecting the abundance and diversity of soil microorganisms (Johnson et al., 2003; Wardle, 2005). But the mechanisms through which plant composition and diversity affect soil communities and trophic levels in soil food webs remain essentially unexplored (Wardle et al., 2003).

Little is known of the importance of microbial diversity in the functioning of soils (Pankhurst et al., 1996; Giller et al., 1997). It has been generally hypothesised that reductions in soil microbial diversity will result in reductions in the functional capability of soils (Giller et al., 1997). In contrast, a decrease in soil microbial diversity does not consistently result in a reduction in the functional diversity of the microbial community (Klein et al., 1986; Atlas et al., 1991). The functional diversity of microbial communities includes the range and relative expression of activities involved in such functions as decomposition of different compounds, nutrient transformations, plant growth promotion and suppression and various soil physical processes influenced by microorganisms (Giller et al., 1997). The diversity of decomposition functions performed by microorganisms, represents one component of microbial functional diversity.

The determination of functional diversity of soil microbial communities has been limited by suitable techniques to assess the immense width of the actual functional diversity in soil microbial communities. Fumigation of soil can kill almost all fungi and most bacteria (Ingham and Horton, 1987). Changes in soil microbial diversity have beenassessed by phospholipid fatty acid (PLFA) profiles, that has provided indicators of changes in community structure.

A popular approach has been to analyze the patterns of substrate use of soil microbial communities in micro–titre wells to assess the soil microbial functional diversity (Garland and Mills, 1991; Insam and Rangger, 1997). It is not clear whether this approach provides a reliable measure of diversity that is representative of the whole soil microbial community because this method probably only assesses the culturable organisms in soils. This studied fraction can be less diverse than the total microbial community in soils (Torsvik et al., 1990). In addition, the expression of microbial functional diversity in vitro is not necessarily directly indicative of the expression of this diversity in situ. A measure of microbial functional diversity in soils can be obtained by measurement of microorganisms catabolism, that gives the patterns of the "*In Situ Catabolic Potential*" (ISCP) of microbial communities, after measurements of respiration responses to a range of substrates (Degens and Harris, 1997). Patterns of ISCP provide a real measure of microbial functional diversity because they provide a direct measurement of substrate catabolism by microbial communities in soils without prior culturing of organisms which is required in the culture–based methods. Patterns of ISCP are not intended to provide a comprehensive measure of microbial functional diversity, but an indicator of the diversity of general microbial functions in soils associated with decomposition of organic matter.

Changes in patterns of ISCP over time may reflect changes in the composition of mineralisable organic matter in soils (Degens and Harris, 1997). However, it is not known to what extent this measure of microbial functional diversity can be influenced by the composition of recent organic inputs into soil. Most of the readily decomposable organic C added to soil is rapidly consumed by microorganisms, thereafter, decomposition is dominated by turnover of the microbial C and slower decomposition of the more recalcitrant organic C (van Veen and Kuikman, 1990; Ladd et al., 1995).

2.3 Alien tree species and allelopathy

Introduction of alien (or exotic) tree species into a country are intentional (deliberate) or unintentional (accidental) (Di Castri, 1989, 1991; Di Castri et al., 1990; Lambinon, 1997) and can be subject to different motives.

For intentional introductions, the motive in most cases is the trade with the tree itself, usually there is an interest on both sides, the exporter and the importer, to introduce this tree species into a country (e.g. by afforestation or reforestation).

Several plant species cannot be established permanently, others just blend into the environment without causing any competition for space and resources or alteration of soil chemistry or water regime. In contrast some species that have been introduced intentionally.

According to the Guiding Principles for the Prevention, Introduction and Mitigation of Impacts of Alien Species (CBD, 2002) of the Convention on Biological Diversity (UNEP, 1992), invasive alien species are non–indigenous organisms that threaten biodiversity (Vitousek, 1997). In many cases invasive plant species can outcompete native species, which detract from the local biodiversity of the area (Faulkner, 2002). In some extreme cases, the invasive plant species can totally eliminate a native population and form a pure stand (Tellman, 2002). If a species has been identified to be invasive or potentially invasive, it has:

- an important *primary consequence*: the reduction of the abundance of keystone plant species compromises development of or succession within an ecosystem.
- *secondary consequences*: effects on plant communities, namely effects on designated environmentally sensitive or protected areas, namely changes in the structure, stability or dynamics of an ecosystem (including further effects on plant species, erosion, water–table changes, increased fire hazard, nutrient cycling, etc.), effects on human use (e.g., water quality, recreational uses, tourism, animal grazing, hunting, fishing), or costs of environmental restoration. Black locust (*Robinia pseudoacacia* L.) has been widely planted all over the world and as revealed a powerful invasive potential. Invasion of exotic species, like Black locust, is widely recognized as one of the major threats to biodiversity and ecosystem stability. Once introduced,

> Black locust expands readily and outcompetes native species. Black locust due to its ability to sybiotically fix nitrogen may have a significant effect on the whole plant community, as ecological processes may be affected by the heavy accumulation of nitrogen in the soil. Such an introduction may have a significant negative impact on nutrient–poor soils, which often are habitats for endangered plant species. Moreover Black locust leaves contain allelopathic compounds that reduce the growth of herbaceous species (Nasir et al., 2005; Fujii et al., 2004) and are involved in resistance to the locust leafminer (Zheng et al., 2003). In forest ecosystem, allelopathic compounds produced by alien tree species are responsible for the number of understory species and the scarcity of their cover (Chou and Leu, 1992; Souto et al., 1994; Yamamoto, 1995).

Allelopathy, from the Greek words *allelon* "of each other" and *pathos* "to suffer", is defined (Molisch, 1937) as encompassing both stimulatory and inhibitory biochemical interactions among plants and microorganisms at all levels of complexity, affecting growth, health, behaviour, or population biology of involved organisms. Thus one plant (including associated microorganisms) may have direct or indirect chemical effects on a neighbour (Rice, 1974). Aldrich (1984) describes two types of allelopathy:

1. *True type*: the release into the environment of compounds that are toxic in the form in which they are produced,
2. *Functional type*: the release into the environment of a substance that is toxic as the result of transformation by micro–organisms.

While according to Aldrich (1984) compounds with allelopathic effects must be concentrated in the leaves, stem or roots rather than in the fruit or flowers, Rice (1984) and Putnam (1985) reported that allelopathic compounds are present in virtually all plant tissue, i.e. leaves, fruit, stems, and roots. Allelopathic compounds are released by such processes as:

- *Volatilization,* viz. release into the atmosphere. This process is significant only under arid or semi–arid conditions. The compounds may be dissolved in vapour and absorbed by surrounding plants, be absorbed from condensates such as dew or may reach the soil and be taken up by roots.

- *Leaching through rainfall*. Dew or irrigation may leach the compounds from the aboveground parts of plants and subsequently deposit them on other plants or on the soil. Leaching may also take place from plant residues. The solubility of the allelopathic compounds will affect their mobility in soil water.
- *Root exudation*. Release from plant roots into the soil environment. Whether these compounds are actively exuded, leached or relased from dead cells sloughing off from the roots is at present not clearly understood.
- *Decomposition of plant litter*. Toxic substances are possibly contained in plant litter and released upon decomposition. They may, alternatively be produced by microorganisms utilizing the litter. At present this is not clear.

Plants allelochemical compounds may exert effects indirectly by their impact on the soil environment. These effects may be mediated by microbial or non–microbial degradation of plant compounds.

In plant–to–plant allelopathy, the more common poisonous effects are visible upon such plant functions as respiration, photosynthesis, water balance and stomatal function, stem conductance of water, xylem element flux, membrane permeability, cell division and development, protein synthesis, and alteration of enzyme activity. When released from a plant, a potential allelopathic compound may first have to undergo some metabolic or environmental alteration in structure before it can become biologically active. The role of allelochemical compounds is mostly interpreted to be a means of plant defense against other plants, competition, defense against pests, or diseases.

How plants interact with each other is pertinent to how communities are organized. When plants react differently to chemical compounds released from particular neighbours, it may have a substantial effect on species coexistence and community composition.

To establish evidence for allelopathy, it is needed to show that the donor plant is capable of accumulating allelochemical compounds in the environment at phytotoxic levels, and that the vegetation pattern is not solely explained by physical (e.g. resource) or biotic (e.g. herbivore) factors (Blum et al., 1999).

The "*Allelopathic Advantage Against Resident Species*" hypothesis or "AAARS" (Callaway and Aschehoug, 2000; Bais et al., 2003; Callaway and Ridenour, 2004; Callaway et al., 2004; Vivanco et al., 2004) suggests that the success of some exotic invasive plant species may be due to the possession of "novel weapons", biochemical compounds that native species have never encountered. The concept "novel weapons" suggests a mechanism for the evolution of increased competitive ability in invasive plants. Thus, invaders possess allelopathic weapons that provide greater competitive advantages in their new habitats than in their original ones.

Different microbial communities are associated with different plant species (Bever, 1994; Grayston and Campbell, 1996; Westover et al., 1997; Priha et al. 1999, 2001; Grayston et al., 2001; Klironomos, 2002), probably due to species–specific rhizosphere biochemical compounds and the addition or removal of particular resources. Substantial evidence suggests that soil microorganisms has important effects on the success of invasive plants (Klironomos, 2002; Mitchell and Power, 2003; Reinhart et al., 2003; Callaway, 2002) and that interactions between plants and microorganisms are based, in part on biochemical compounds, even if yet there are no clear links between new unidentified biochemical compounds, soil microbial communities, and invasive success (Callaway and Ridenour, 2004).

Although the study of allelopathy involving plants in agricultural and horticultural systems has a long history, research on allelopathy in forested ecosystems is rather recent. In forested ecosystems allelopathic effects can be induced by tree canopy on same species (by auto–toxicity) as well as on other tree species causing forest decline. Trees and understory plants can also affect each other allelopathically causing an overall decline in species richness and diversity. These changes happen at varying spatial and temporal scales causing local to ecosystem level differences in forest structure and composition.

2.4 The importance of forests within the Kyoto Protocol

After the Kyoto meeting in 1997, the impact of forest management on nutrient cycling and C and N pools has received special attention.

Article 3.3 of the Kyoto Protocol establishes that all countries must calculate the net changes in greenhouse gas emissions by sources and removals by sinks. Changes in net greenhouse gas emission may result from direct human–induced land–use change and forestry activities limited to afforestation, reforestation and deforestation since 1990. Deforestation, is the direct human–induced conversion of forested land to non–forested land.

Often “afforestation” is confused with “reforestation”, because, commonly, the two terms are used to indicate plantation of forest trees, but the meaning is completely different:

- *Afforestation*, is the direct human–induced conversion of land that has not been forested for a period of at least 50 years (e.g. severely burned stands and degraded forest stands where trees are not present) to forested land through planting, seeding and/or the human–induced promotion of natural seed sources.

- *Reforestation*, is the direct human–induced conversion of non forested land to forested land through planting, seeding and/or the human–induced promotion of natural seed sources, on land that was forested but that has been converted to non–forested land (i.e. harvest–field, agricultural land).

Since the dawn of the industrial revolution many countries have seen rapid decline in forest cover. Hence, both governments and non–governmental organisations (NGO's) have been directly promoting afforestation and reforestation programs. Afforestation and reforestation are recognized as a major sink for carbon sequestration (FAO, 2004).

Several studies have estimated the contribution of afforestation to the global C cycle at both regional (Sharpe and Johnson, 1981; Armetano et al., 1982; Berg and McClaugherty, 2008; Akselsson et al., 2005) and global scales (Johnson and Sharpe, 1983; Houghton et al., 1983; Anonymous, 1999; Callesen et al., 2003). Most of the available information on global carbon budgets gives projections on C accumulation by vegetation following afforestation (e.g. Grigal and Berguson, 1998); little information is included on associated changes in soil C (Scott et al., 1998). It is known that forest soils hold about 40% of all belowground terrestrial C (SOM, soils, litter, and roots) (Dixon et al., 1994; Huntington, 1995). Accumulation rate of soil C was calculated under forests on young volcanic soils (Wilde, 1964; Vitousek et al., 1983; Schlesinger, 1990), as well as following a rehabilitation of a mine site (Smith et al., 1997), and following mudflows (Dickson and Crocker, 1953).

After the governments commitments at the Kyoto convention, Italy is expected to meet a reduction of 6.5% of the 1990 levels of the net green house gas (GHG) emissions (the sum of all green house gases transformed to C equivalents) in the first commitment period, 2008–2012. The Protocol provides a basic framework for the inclusion of a limited number of carbon sink activities to reduce net national changes of GHG emissions.

3. AIM OF THE STUDY

The concern for the effects of green house gases, among them CO_2, has increased the interest in the dynamics of C in soil and, today, soil organic matter (SOM) in forest ecosystems receives more attention.

Forest ecosystems represent an important C storage pool (Sedjo, 1992; Dixon et al., 1994; Houghton et al., 2001). SOM in forest soil, both in the organic and mineral layers, is a sink for C (Berg et al., 1995; Berg et al., 200X), and the accumulation rate of carbon may depend on the chemical composition of the litter that is shed from the plants (Berg et al., 1996; Berg and McClaugherty, 2008). Forest management and forest disturbances (i.e. wildfire, deforestation, clearcutting, site preparation) may have a profound effect on C cycling, mineralizing stored carbon. Without disturbances, the amount of organic matter continues to increase in soils over centuries or even millennia (Jenny, 1980; Wardle et al., 1997).

In forest ecosystem, allelopathic chemical compounds produced by alien tree species might inhibit soil microbial activity with consequent effects on storage rates of SOM and ecosystem nutrient cycling (Souto et al., 2000a; 2000b; Klinke et al., 2003; Kalinova et al., 2007). Until know little attention has been paid to this topic that is worthy of investigation because alien species are largely used for afforestation of poor soils or easily invade bare soils.

Forest stands at Mount Vesuvius are a convenient study area to investigate C sequestration in soil. Chronosequences of tree plantation occur on volcanic substrate of know age; moreover different species of tree have been used for afforestation including the alien invasive species *Robinia pseudoacacia*. Thus it is possible to study carbon sequestration in the soil as related to the age of the colonised stand and to type of tree cover.

Five forest stands were selected: three of them constituted a chronosequence (36, 66 and 96 years) of Stone pine; the other two were coeval (36 years) plantations of Corsican pine and Black locust.

The aims of the present study started from following questions:

1. How does C sequestration differ between two adjacent stands, with the same climate, soil age, plantation age and different plant cover?
2. How does C sequestration vary in a chronosequence of three pine stands, that is in woodlands of same pine species but different soil and plantation age?
3. What kind of information about C sequestration rate when we compare all five stands?

4. Is there an allelopathic effect on soil microbial activity in a Black locust litter? May the allelopathic effect increase C sequestration in soil organic matter?

To answer questions 1–3 measurements were performed of a) litter fall, b) carbon stocks in organic and mineral soil layers, c) chemical properties of litter and humus related to the resistance to decomposition.

To investigate the occurrence of allelopatic compounds in the litter of Black locust and their effects on soil metabolism and SOM dynamics, laboratory and field experiments were performed on Corsican pine soil.

4. MATERIAL AND METHODS

4.1 Study area

The Vesuvius National Park, in southern Italy, officially designated on June 5th 1995, represents an important area to safeguard. Mount Vesuvius is the only active volcano in continental Europe, the most populated (20 municipalities with a total of 400,000 residents) and is also the most extensively studied volcano on Earth. The current shape of the volcano is the result of the continual alternation between “explosive” and “effusive” type eruptions, which, producing, pyroclastic deposits and lava, respectively, have partly destroyed the most ancient volcano, Mount Somma, within which the Gran Cono of Mont Vesuvius later formed. The two structures as a whole are known as the Somma–Vesuvius volcanic complex (Fig 1).

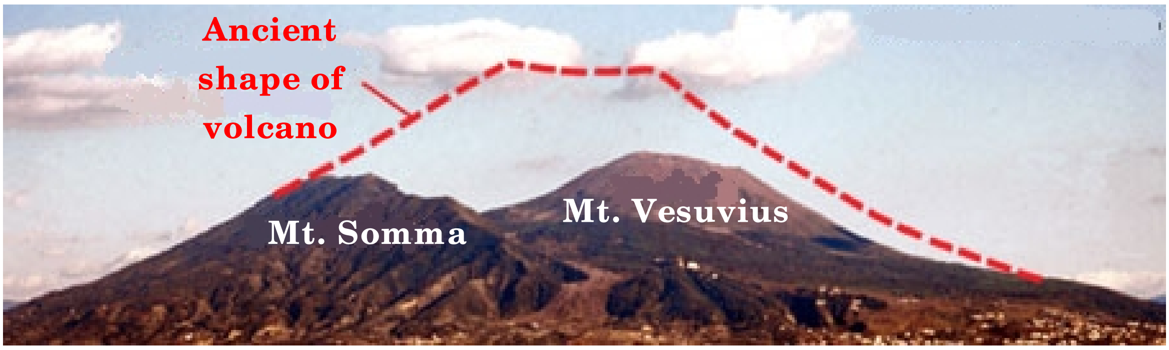


Figure 1. Original profile of ancient Somma volcano (Cioni et al., 1999). Mount Somma is that remaining of northern side of ald shape.

The Vesuvius side has a characteristic Mediterranean–type vegetation (di Gennaro et al., 2002). Colonisation of the lava by vegetation happens thanks to a greyish–silver lichen called *Stereocaulon vesuvianum* Pers., which prepares the ground for settlement of other pioneering species, and eventually maquis or scrub is formed. The Somma side instead is damper and is covered by mesophyll forest vegetation, with a prevalence of mixed woodlands of Chestnut, Oak, Alder, Maple and Ilex trees. Over nine hundred plant species have colonised the volcanic complex over time; the current composition of its flora includes 610 species, among which 18 endemic ones of great interest, such as Perpetuin (*Helichrysum litoreum* Guss.). Another interesting feature is the presence of twenty species of orchids and small nuclei of Birch (B*etula pendula* Roth.), remaining evidence of the mesophile woodland that once covered the slopes of the volcano. Different species have alien origin, some ones are naturalized, as e.g. pine species (*Pinus pinea* L., *Pinus pinaster* A., *Pinus nigra* Arn.), also known as Stone pine, Maritime pine and Corsican pine, respectively; other alien species have shown their invasive and opportunistic behaviuor, like *Ailanthus altissima* (Mill.) S. (Tree of heaven) and *Robinia pseudoacacia* L. (Black locust).

The Vesuvius National Park is situated in the province of Naples (southern Italy), and extends 8,482 hectares (Fig. 2). The highest point of Mount Vesuvius reaches 1.282 metres above sea level (m.a.s.l.), while Mount Somma reaches a height of 1.132 m.a.s.l. with Punta Nasone.

Figure 2. The Vesuvius National Park (southern Italy), extends 8,482 hectares across the territory of 13 municipalities.

4.2 Site descriptions and plot size

Five mature forest stands (Table 1) were localized:

1. Two adjacent stands in Atrio del Cavallo on the northern side between Mount Vesuvius and Mount Somma: one was a Black locust (*Robinia pseudoacacia* L.) monoculture (1142 m^2), the other one a Corsican pine (*Pinus nigra* Arn. var. *corsicana*) monoculture (1640 m^2). Both were 36 year old in 2006 (Fig. 3).

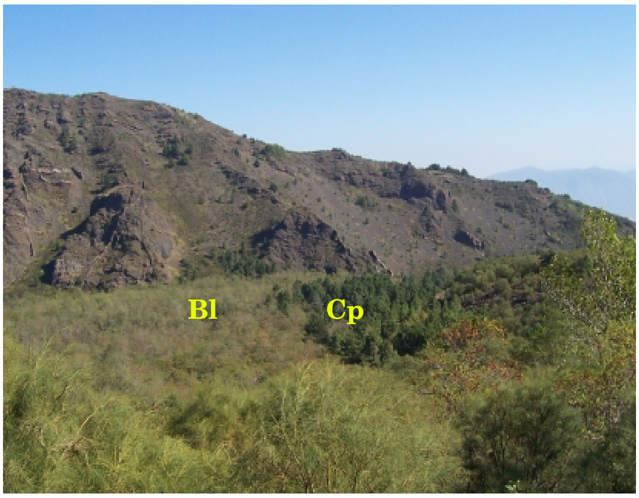


Figure 3. Atrio del Cavallo in the northern side of Mount Vesuvius. To the left a Bl=Black locust (*Robinia pseudoacacia* L.) monoculture, and to the right a CP=Corsican pine (*Pinus nigra* Arn. var. *corsicana*) monoculture. Both are 36 years old.

2. Two stands with Stone pine (*Pinus pinea* L.) monocultures in Piano delle Ginestre on the southern side of Mount Vesuvius: a 36 year old (4140 m^2) (Fig. 4) and another 66 year old in 2006 (2477 m^2) (Fig. 5).
3. One Stone pine monocolture in Cancello Mangano on the most southern side of Mount Vesuvius: 96 year old (in 2006) (5370 m^2) (Fig. 6).

The soils of all stands are molli–vitric andosol (calcaric), with moderately coarse texture and good oxygen availability (di Gennaro et al., 2002). The climate data based on records from the Gran Cono meteorological station at 1256 m a.s.l. inside Vesuvius National Park and collected from 2003 to 2008. Thus we obtained an average temperature of 9.0°C and an annual precipitation of 640.6 mm.

In the table 1 are given some main characteristics of the study sites.

Table 1. Some main characteristics of the five forest stands with monocultures located on Mount Vesuvius.

Site name (Altitude)	**Lat/Long**	**Tree species** (Year planted)	**Understory**	**Litology** (age)	**Soil**	**Slope**
ACR (880)	40°49'53''N 14°26'13''E	*Robinia pseudoacacia* (1970)	*Rubus fruticosus*, *Graminaceae*, *Leguminosae*	Lapillus (1944)	Sandy	Flat
ACP (885)	40°49'43''N 14°26'20''E	*Pinus nigra* (1970)	*Genista aetnensis*, *Quercus ilex*	Lapillus (1944)	Sandy	Softly inclined
BF (631)	40°48'40''N 14°24'53''E	*Pinus pinea* (1970)	*Spartium junceum*, some *Q.ilex*, *R. pseudoacacia*	Lava (1867)	Sandy with gravel	Moderately steep
PG (550)	40°48'34''N 14°24'26''E	*Pinus pinea* (1940)	*Q.ilex*, *R. pseudoacacia*	Lava (1867)	Sandy with gravel	Wavy
CM (350)	40°47'51''N 14°26'30''E	*Pinus pinea* (1910)	*S.junceum*, *Cistus incanus*, *Q.ilex*, other *Leguminosae*	Lava (1906)	Sandy	Steep

Figure 4. Piano delle Ginestre. Details of litter trap in the 36–year–old Stone pine (*Pinus pinea* L.) monoculture on the southern side of Mount Vesuvius.

Figure 5. Piano delle Ginestre (PG). Details of litter trap in the 66–year–old Stone pine (*Pinus pinea* L.) monoculture on the southern side of Mount Vesuvius.

Figure 6. Cancello mangano (CM). Details of litter trap in the 96–year–old Stone pine (*Pinus pinea* L.) monoculture on the most southern side of Mount Vesuvius.

4.3 The Forest stands

4.3.1 *Robinia pseudoacacia* L.

Robinia pseudoacacia L., also known as Black locust, is an early successional plant, preferring full sun and well–drained soils. It invades dry and sandy prairies, oak savannas, and upland forest edges and is commonly found in disturbed areas such as abandoned fields, in degraded woods, and at roadsides (Weiseler, 1998). It is native to the southeastern United States mainly on the lower slopes of the Appalachian Mountains and with separate outliers north along the slopes and at forest edges in southern Illinois, Indiana, and Missouri.

Outside of its native range, Black locust has become naturalized throughout the United States, has been reported in a few Canadian provinces (Converse, 1984) and reported as invasive in Cyprus, Korea, and Italy.

Once introduced, Black locust expands readily into areas where the shading canopies reduce competition from other (sun–loving) plants. Wieseler (1998) states that Black locust reproduces vigorously by root suckering and stump sprouting to form groves (or clones) of trees interconnected by a common fibrous root system. Dense clones of Black locust create shaded islands with little ground vegetation. Further, the large, fragrant blossoms compete with native plants for pollinating bees. According to OPLIN (2001), Black locust has been used for ornamental purposes and is planted on reclaimed land and to control erosion.

4.3.2 *Pinus nigra* Arn.

Pinus nigra Arn., grows widely throughout southern Europe from the eastern half of Spain, southern France, and Italy to Austria; south throughout the Balkan Peninsula; east to southern Ukraine and south to Turkey; and on the islands of Cyprus, Sicily, and Corsica, with outliers in Algeria and Morocco (Mirov, 1967). It is found at elevations ranging from sea level to 2,000 m, most commonly from 250 to 1,600 m.

The species is divided into two subspecies, each of which is further subdivided into three varieties:

Pinus nigra subsp. *nigra* in the eastern part of its extention, from northeast and central Italy, to the Crimea and Turkey:

- *Pinus nigra* subsp. *nigra* var. *nigra* which is well–known as Austrian pine
- *Pinus nigra* subsp. *nigra* var. *caramanica* or Turkish black pine
- *Pinus nigra* subsp. *nigra* var. *pallasiana* or Crimean pine

Pinus nigra subsp. *salzmannii* in the western part of its extention, from south Italy to south France, Spain and north Africa:

- *Pinus nigra* subsp. *salzmannii* var. *salzmannii* or Cevennes black pine
- *Pinus nigra* subsp. *salzmannii* var. *corsicana* (syn. subsp. *laricio*) or Corsican pine, Calabrian pine (grown in Atrio del Cavallo stand)
- *Pinus nigra* subsp. *salzmannii* var. *mauretanica* or Atlas mts black pine

Although the wood has a relatively larger proportion of sapwood to heartwood and thus requires a long rotation, it is used extensively throughout the Mediterranean region for general construction work, fuelwood, and other purposes for which pine timber is needed (Dallimore et al., 1966).

4.3.3 *Pinus pinea* L.

Pinus pinea L. or Stone pine has also been called Italian Stone pine, European nut pine, Umbrella pine (not to be confused with the Japanese umbrella–pine) and Parasol pine. It has also occasionally been listed under the invalid name *Pinus sativa*. The (Italian) Stone pine (or Umbrella pine) is a species native of the Mediterranean region. It has been cultivated extensively for at least

6,000 years because of its edible seeds. Besides being cultivaed for its seeds it is also a widespread horticultural tree.

The original range for Stone pine encompasses Spain, Portugal, and North Africa including major parts of the Sahara Desert, especially Morocco, Algeria, Tunisia, and Libya, when this area had a more humid climate. It has been cultivated and often naturalised throughout the Mediterranean region, for so long that it is often considered native for the whole region. Since about 1700 AD it has been introduced to other areas with Mediterranean climate and is now naturalised in South Africa, where it is listed as an invasive species, and commonly planted in California, Australia, and western Europe as far north as to southern Scotland.

4.4 Experimental approaches

4.4.1 Litter fall

Litter traps were laid out randomly. The number of replicate traps was 10 in all stands, except in the 36 year–old Stone pine stand which had 9 traps. Each litter trap had a nominal sampling area of 0.79 m^2 and normally mounted at a height of c. 1 m above ground (see Fig. 6). The polyester net used had a mesh size of c. 1 mm. Litter was sorted into two fractions; foliar litter and a composite fraction consisting of all other components such as cones (legumes for Black locust), branches, and bark. The fractions were dried untile dryness, mostly at 50°C. After drying, normally the foliar litter fraction and that made up of other components were weighed individually.

The litter samplers used at all sites could be expected to give reliable values for foliar litter which is more evenly distributed throughout the stands during litter fall, whereas litter of e.g. bark, cones, and branches is sampled less reliably using that method (cf. Flower–Ellis 1985).

We collected litter fall from the Blackberry bushes inside the Black locust stands, using 20 rectangular litter traps (20 traps; each of 0.15 m^2) placed under the bushes, at the base of brambles (Fig. 7).

Litter fall was sampled for 2 years from May 20, 2006 to July 21, 2008 and an average value was calculated and used. The sampling frequency varied among the plots. Traps were emptied five times a year in the Atrio del Cavallo stands and four times a year in the three Stone pine stands. Thus, the possibility of some decomposition between samplings can not be excluded.

Figure 7. Litter traps under Blackberry bushes on the northern side of Mount Vesuvius. Details, inside the Black locust stand.

4.4.1.1 Basal area, a new approach for litter fall study

A traditional way of presenting litter fall data from trees is to use the unit kg ha^{-1} yr^{-1}, not considering stand density, which can vary naturally, e.g with latitude, soil conditions, tree species and with forest management. One possibility to improve this would be to use a direct measure of the number of litter–producing trees per hectare (number of stems per hectare). Another measure of live tree biomass is the basal area (BA), given as stem surface per hectare, with the surface measured at 130 cm stem height. The normally used unit for basal area is m^2 ha^{-1}. In the present study I evaluate and suggest a new approach to determine and present foliar and total litter fall (LF and TLF) data. To this purpose I have created a quotient of litter fall and basal area (LF/BA and TLF/BA) with the unit kg m^{-2}.

4.4.2 Sampling of organic and mineral layers

To collect the soil organic matter in the organic layer I used a square sampler of 400 cm^2 excluding live grass and other live plants (Fig. 8). Twenty spots were sampled in each stands. Litter and humus were separated in the laboratory.

Figure 8. A quadratic sampler of 400 cm^2 (20 x 20cm) for collecting the material in the organic layer in the stands studied: details in Corsican pine stands (to the left) and in Black locust stand (to the right).

To collect the soil organic matter in the mineral layer, initially, an overview inspection was carried out and the mineral soil samples of 1 cm of height were been taken until 15 cm of depth, when possible (below 15 cm stones or rock made sampling impossible). After the mineral soil was sampled at 20 spots in each stand, when possible. We used a corer with the surface of 25 cm^2 (Fig. 9). Cores were sampled to a maximum depth of 15 cm. In the laboratory the collected cores were divided into 5 cm sections before sieving (2 mm mesh).

Figure 9. The mineral soil was sampled until a depth of 15 cm when possible using a corer with the surface of 25 cm^2.

The organic and mineral fractions were dried until dryness, mostly at 75°C. After drying, the samples were weighed individually, and homogenized in a Fritsch pulverisette for CN analysis.

Samplings of organic and mineral layers were carried out from March 29, 2007 until May 07, 2008.

4.4.3 To assess allelopathic effects of Black locust litter on soil microbial community

Laboratory and field experiments was performed. In the laboratory, far decomposed litter from the forest floor and newly shed litter from litter traps placed inside the Black locust stand (Fig. 10), were used to make aqueous extracts at different dilutions. Known amounts (10.0 g) of dry litter, both decomposed and newly shed, were suspensed in distilled water (100 ml) for 5 hours. Samples were centrifuged (4000 rpm for 10 min), sterilized by microfiltration with 0.22 µm pore filter, diluted, with distilled water, twice, 100, 10,000 and 100,000 times and stored at –20°C until bioassay.

Samples from the 0-5 cm layer of the mineral soil of the Black locust and Corsican pine standswere used to estimate whole microbial activity in these soils. Aqueous extracts of Black locust litter at different dilutions were applied to Corsican pine and Black locust soil samples to reach 55% Water Holding Capacity (WHC). Soil samples were incubated at 25°C and basal respiration, microbial biomass, carbon endogenous mineralization and catabolic evenness were measured and calculated after 12d.

Figure 10. Litter trap in the Black locust stand in Atrio del Cavallo on the northern side of Mount Vesuvius.

In field, in Corsican pine stand, after removal of needle litter and humus in delimited area by microcosms (50x50cm), 1,5 Kg litter (average estimate of total litter which were removed) are put into microcosms: i) 9 microcosms with needle litter (Control); ii) 9 microcosms with far decomposed Black locust litter (DL); iii) 9 microcosms with newly shed Black locust litter (FL) (Fig. 11). In total we have 27 microcosms. Basal respiration, microbial biomass, active, total fungal biomass, microbial metabolic quotient mineralization and catabolic evenness were measured and calculated after 55d and 244d.

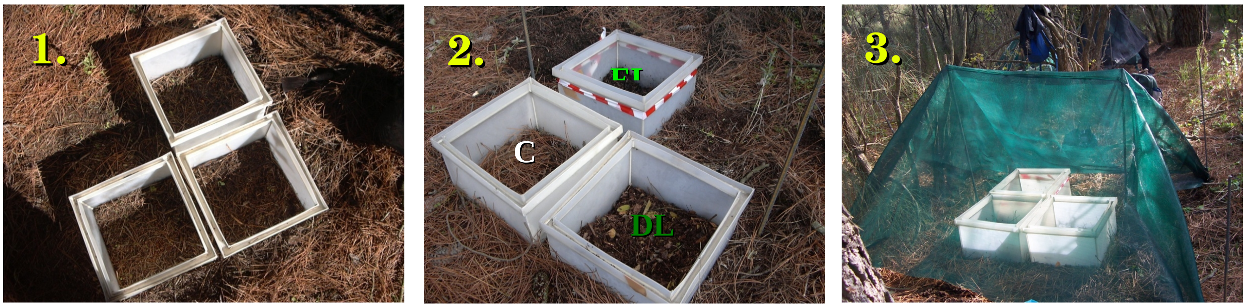


Figure 11. Different steps in the field experiment in the Corsican pine stand: 1. Removal of needle litter and humus and placement of microcosms; 2. Microcosms were filled with i) needle litter (control), ii) newly shed Black locust litter, iii) far decomposed Black locust litter; 3. Protection of microcosms against new litter input.

The laboratory analysis started at the end of April 2006, while field experiments started in October 2007 and finished in July 2008.

4.5 Analysis of ash and calculation of soil organic matter

Samples of 1.0 g of oven–dry sieved soil (2mm mesh) were weighed in small ceramic cups and burned in a muffle furnace at 550°C for 2 hours (Allen, 1989).

After that, ashes were placed in a desiccator and weighed. Soil organic matter content, expressed as percentage of total soil mass, is given by:

$$\text{Organic matter} = ((\text{mass}_{\text{dry soil}} - \text{mass}_{\text{dry ash}})/ \text{mass}_{\text{dry soil}}) \times 100$$

4.5.1 Determination of compounds/groups of compounds in SOM

Solid–state ^{13}C Nuclear Magnetic Resonance (NMR) with cross polarization and magic angle spinning (CP/MAS) has developed over the last two decades to become one of the most useful techniques in the non–destructive study of organic matter in whole soils (Wilson et al., 1981; Kögel–Knabner et al., 1988; Piccolo, 1993; Zech et al., 1994; Preston, 1996; Miltner et al., 1996; Conte et al., 1997; Fabbri et al., 1998; Schmidt et al., 2000; Salloum et al., 2002; Baldock and Smernik, 2002; Keeler et al., 2003; Ussiri et al., 2003 Certini et al., 2004; Schönig et al., 2005). In contrast to many other analytical methods, solid–state NMR spectroscopy does not depend on the solubility of the sample, thereby allowing the examination of insoluble soil fractions as well as bulk soil samples. Unfortunately, such samples often contain little organic matter and considerable amounts of mineral and paramagnetic compounds, which decrease the sensitivity of solid–state ^{13}C NMR. The resulting spectra typically suffer from poor resolution and low signal–to–noise ratios. ^{13}C CPMAS spectra of soil samples (Fig. 12) show peaks in the whole range of chemical shifts between 0 and 200 ppm, with overlapping peaks due to the presence of high numbers of different compounds in the SOM samples. Neverthless it was possible to characterize functional groups in SOM spectra. Thus, we distinguish four zones that correspond to ^{13}C chemical shifts (Skjemstad et al., 1996) (Tab. 2), and belong to characteristic functional groups of SOM.

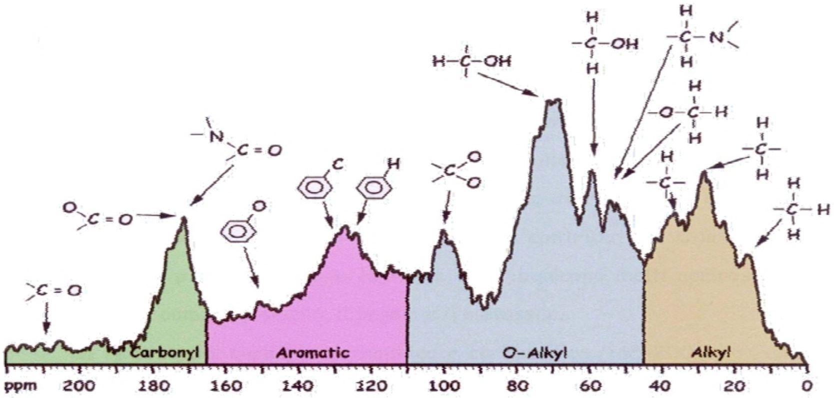


Figure 12. ^{13}C NMR of SOM divided into spectral regions with some signals attributed.

Table 2. The main four zones corresponding to ^{13}C chemical shifts in ^{13}C CPMAS NMR spectra.

Region	Carboxylic C	Aromatic C	O – Alkyl C	Alkyl C
ppm	165 – 190	110 – 165	45 – 110	0 – 45

Litter, humus and soil samples, dried and homogenizated in a Fritsch pulverisette, were analysed, by colleagues of Environmental, Plant and Soil Science Department at CERMANU, with a Bruker AV300 Spectrometer (Fig. 13).

Soil carbon and nitrogen were determined by dry combustion (900°C) using a

CNS analyzer (Elemental Analyser, Flash 112 Series EA) (Fig. 14), after milling (Frisch Pulverisette).


Figure 13. Bruker AV300 Spectrometer of CERMANU, the NMR laboratory of the department of Environmental, Plant and Soil Science (Univ. of Naples–Portici).

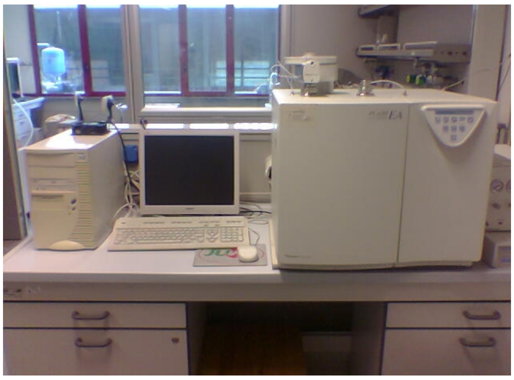


Figure 14. Elemental Analyzer, Flash 112 Series EA in the laboratory where I carried out my PhD iter.

4.6 BIOLOGICAL ANALYSIS

I measured the microbial activity directly by assessing the variation (evenness) in catabolic response profiles (Degens and Harris, 1997; Degens, 1998a,b; Degens et al., 2000). Microbial catabolic evenness was determined by measurement of the short–term respiration responses of soils after addition of solutions of 25 different simple organic compounds (Degens and Harris, 1997; Degens, 1998b). The various substrates were added as 2 ml solutions into vials containing fresh soil, equivalent to 1 g of dry soil, after which they were sealed with Vacutainer stoppers. A no–substrate control treatment, in which only deionised water was added to the soils, was also conducted to determine whether each substrate caused a respiration response above basal respiration. CO_2 efflux from each sample was measured using a gaschromatograph with an electron capture detector (GC 8000, Fison Instruments) (Fig. 15) after incubation of the bottles for 4 h at 25°C (Degens et al., 2000). During the incubation, all bottles were vigorously mixed once using a vortex mixer for 15 to 20 s, once 2 h after substrate addition and once before sampling the head space gas for CO_2 analysis. The substrates used in the assay were: eight amino acids (L–arginine, L–asparagine, D–glucosamine, L–glutamic acid, L–glutamine, L–histidine, L–lysine, L–serine), two carbohydrates (D–glucose, D–mannose), and 15 carboxylic acids (L–ascorbic acid, citric acid, fumaric acid, gluconic acid, a–ketobutyric acid, a–ketoglutaric acid, a–ketovaleric acid, DL–malic acid, malonic acid, pantothenic acid, quinic acid, succinic acid, tartaric acid, uric acid, and urocanic acid). These substrates were those giving the greatest discrimination between soils (Degens and Harris, 1997; Degens, 1998b). All solutions were adjusted to a pH between 5.8 and 6.0 before addition to the samples (Degens, 1998b).

Figure 15. Gas–chromatograph with an electron capture detector (GC 8000, Fison Instruments) in the laboratory where I carried out my PhD.

As outlined above, catabolic diversity is composed of two components: richness and evenness. Richness in this case was the number of substrates used by the microorganisms, whereas evenness was the variability of substrate use across the range of substrates tested. For all soils tested here, richness was not different between the soils because there was a response (above the no–substrate treatment) to all substrates. It was therefore appropriate to concentrate on catabolic evenness. Catabolic evenness (E) was calculated using the Simpson–Yule index:

$$E = 1/\Sigma p_i^2$$

where p_i is a value given from the respiration response to individual substrates as a proportion of total respiration activity induced by all substrates for a soil (Magurran, 1988). Using this formula, the maximum achievable evenness (where all substrates respond equally) was 25, which is the sum of the response of all substrates. Similar patterns were also obtained by other evenness indices such as the coefficient of variation of substrate responses or deriving evenness from the Shannon–Weaver index (Magurran, 1988).

Microbial biomass expressed as C was determined by:

$$\mu g\ C\ g^{-1} soil = 50.4 \times \text{respiration value } (\mu L\ CO_2\ g^{-1} soil\ h^{-1})$$

where 50.4 is a constant value.

4.7 STATISTICAL ANALYSIS

The statistical analyses were performed using Sigma Stat 3.0. All mean values were calculated as arithmetic mean and in the graph and tables are reported standard errors. The differences between organic layer and mineral soil for organic matter and content of C and N were evaluated by one way ANOVA followed by Dunn Test.

Two-way analysis of variance (ANOVA) was performed to test the effects of litter types and time collection on microbial biomass, fungal biomass and microbial activity.

Normal distribution of data was checked before running correlation analyses (Normality test).

Correlations between litterfall over basal area and stand age were analysed using the Spearman's coefficient .

5. RESULTS AND DISCUSSION

5.1 Litter fall and basal area

Litter fall was different among the stands and the reasons to this are analyzed. The litter fall data for two years in all stands are shown in Fig 16.

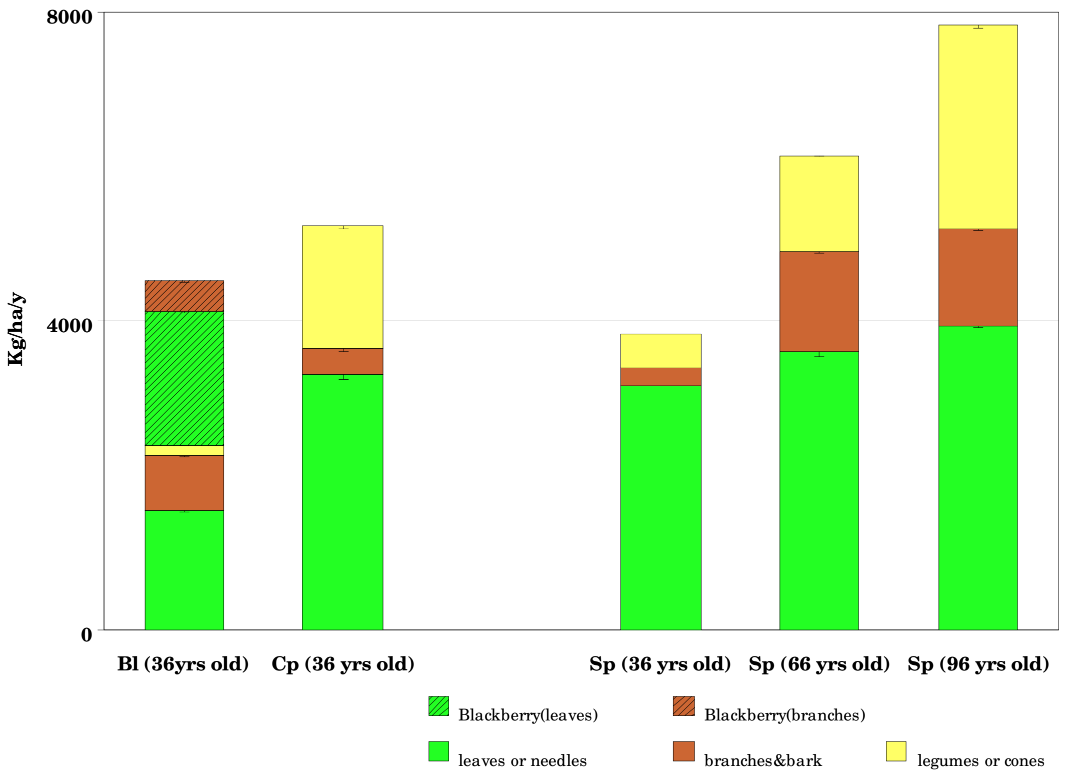


Figure 16. Litter fall in the studied five stands on Mount Vesuvius. Bl = Black locust with an understory of Blackberry; Cp = Corsican pine; Sp = Stone pine. Data are based on litter collections from May 20, 2006 to July 21, 2008.

Of the two adjacent and even–aged stands with Black locust and Corsican pine, on the same soil the results show higher litter fall in the coniferous stand than in the deciduous one ($p < 0.05$). Also when litter fall data from the Blackberry bushes were added to those of Black locust, the combined litter fall was lower than in the Corsican pine stand ($p < 0.05$) (Fig.16).

For the three stands of Stone pine (the three columns to the right, Fig. 16) we may see an increase from the 36 year old stand to that 96 years old ($p <0.05$), so as reported in a Scots pine cronosequence (Berg and Laskowki, 2006). Across the cronosequence, an overall trend in litter fall composition was noted: from the highest proportion of the needle component in the youngest stand to successively lower proportion of needles in the older stands and increasing proportion of cones and branches/bark. In this cronosequence cones develop and are dropped as trees reach their physiological maturity: 11% for 36 years old stand, 20% for 66 years old one and 34% for 96 years old one, while branches/bark and cones start falling later: 6%, 21%, 16%, respectively. For needles we have, respectively: 83%, 58% and 50%. These latter percentage reflect a physiological behaviour from younger stand to older one (Berg and Laskowski, 2006), and is better appreciated if we consider an index of tree biomass, as the basal area (BA) (Tab. 3), and relate it to litter fall (Fig. 17).

Table 3. Foliar Litter Fall (LF). Total Litter Fall (TLF = leaves or needles + branches and bark + legumes or cones). Basal Area (BA) and number of trees per hectare (n°/ha) for the five stands on Mount Vesuvius.

	LF (Kg/ha/y)	TLF (Kg/ha/y)	BA (m^2/ha)	stems/ha
Black locust (36 yrs old)	1544.5	2395.7	26.4	2091
Corsican pine (36yrs old)	3313.2	5234.0	24.9	350
Stone pine (36 yrs old)	3170.0	3827.8	31.0	1232
Stone pine (66 yrs old)	3593.0	6143.5	49.7	916
Stone pine (96 yrs old)	3940.4	7830.9	54.0	361

Table 3 reports data for foliar litter fall (LF), total litter fall (TFL) and the basal area (BA). The quotient of foliar litter fall and basal area (LF/BA) or total litter fall over basal area (TLF/BA) gives a more appropriate measurement of the amount of litter that falls from trees as related to their biomass, than that got considering just the number of trees present in the area.

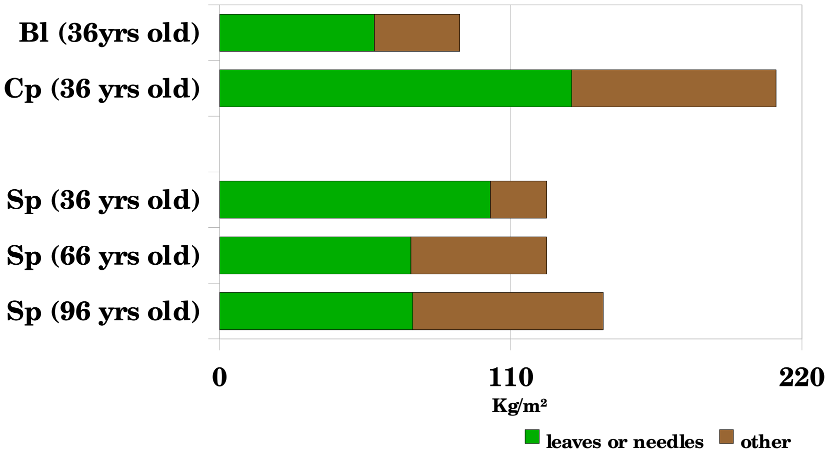


Figure 17. Litter fall (LF) over Basal Area (BA) for the five stands on Mount Vesuvius. "Other" = branches and bark + legumes or cones. Bl = Black locust; Cp = Corsican pine; Sp = Stone pine.

In Fig. 17, considering the new quotient LF/BA, we can appreciate the amount of needles related to biomass: the most productive among the five stands are the two 36 years old stands of Corsican and Stone pine ($p < 0.05$). If we consider branches/bark plus legumes or cones, or in other words calculating the total litter fall (TLF) over basal area (BA), we may see a trend similar to that observed in Fig. 16. The 66 years old Stone pine stand and the 36 year old one have similar values; higher values are reached by Corsican pine (36 years old) probably because of higher water and nutrients availability and a more suitable substrate, lapillus. The lava at the Stone pine stands is more resistant than lapillus to weathering thus giving a more nutrient poor soil retarding plant growth. Unfortunately any comparison for climatic conditions is possible between pine stands because only climatic data of Gran Cono station, at about the same altitude of Corsican pine stand, are available.

5.2 Soil organic matter

A first survey of the study stands was obtained through the determination of soil organic matter content from the top mineral soil up to 15 cm depth, separately for each one-centimeter layer (Fig. 18). As expected (Yang et al., 2004; Sidari et al., 2005) organic matter content decreases along the profile. In the Black locust soil more organic matter is accumulated in the first 5 cm depth, probably due to its recalcitrance (Kononova, 1975; Schlesinger, 1977; Sequi, 1989; Trumbore, 1993).

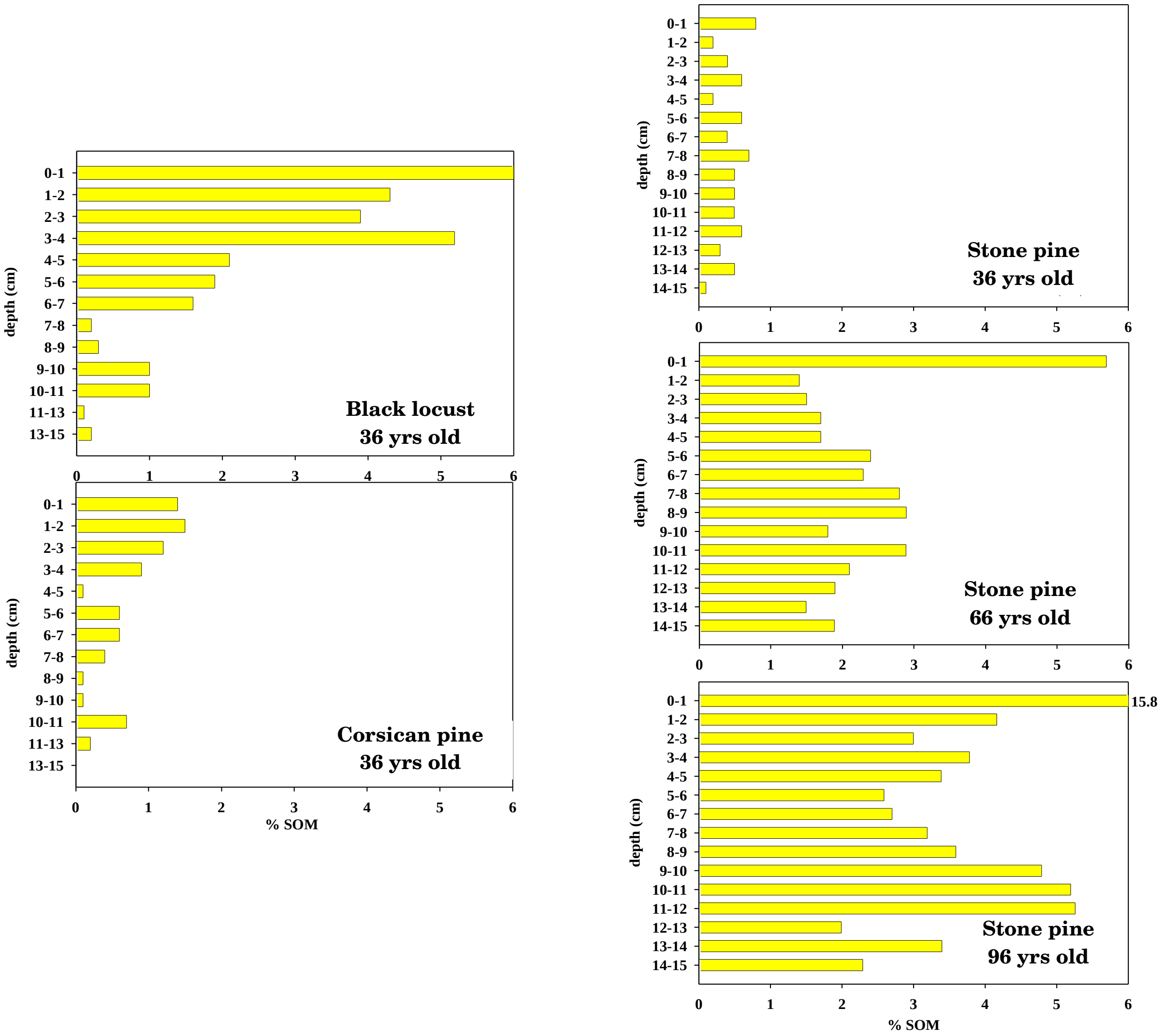


Figure 18. Soil organic matter as percent of soil dry weight for each one-centimeter layer up to 15 cm depth (when possible) for all study stands on Mount Vesuvius.

Consistently with the data of Ovington (1959) and Wardle et al. (1997), in the Stone pine cronosequence the accumulation of carbon in the soil with stand age is evident. However, when comparing all study stands the trend becomes less clear probably due to differences among stands for climatic (Jenny, 1980) and edaphic conditions and for litter quality.

5.3 Concentrations of organic matter, carbon and nitrogen in five stand

The results of determinations of organic matter, carbon and nitrogen content in organic and mineral layer of the two even aged, adjacent stands highlight the differences between the two soils under a different plant cover as reported in Table 4.

Table 4. Concentrations (as percent of dry total soil mass) of Organic Matter (OM), Carbon (C) and Nitrogen (N) in organic layer and in mineral layer (at different depth) in two adjacent stands on Mount Vesuvius . Values are means with standard error in parentheses. C/N is the ratio Carbon to Nitrogen.

	Black locust (36 yrs old)				**Corsican Pine (36 yrs old)**			
	OM	C	N	C/N	OM	C	N	C/N
Organic layer								
Litter n = 20	79.46 (± 3.11)	41.99 (± 0.80)	2.23 (± 0.08)	18.8	92.14 (± 2.12)	46.88 (± 1.00)	1.26 (± 0.08)	37.2
Humus n = 20	67.38 (± 2.58)	31.87 (± 0.09)	2.34 (± 0.09)	13.6	70.27 (± 5.64)	37.04 (± 2.29)	1.41 (± 0.11)	26.3
Mineral layer								
0-5 cm n = 18-20	7.70 (± 1.79)	3.37 (± 0.50)	0.52 (± 0.06)	6.5	6.77 (± 1.73)	2.32 (± 0.37)	0.31 (± 0.37)	7.5
5-10 cm n = 18-20	1.80 (± 0.36)	0.75 (± 0.23)	0.23 (± 0.05)	3.3	1.67 (± 0.16)	0.86 (± 0.20)	0.16 (± 0.02)	5.4
10-15 cm n = 14-15	1.66 (± 0.54)	0.26 (± 0.12)	0.07 (± 0.04)	3.7	1.61 (± 0.16)	0.49 (± 0.08)	0.12 (± 0.02)	4.2

n = number of samples.

There are significant ($p < 0.05$) differences between the two stands for organic matter (Tukey test), carbon (Dunn's test) and nitrogen (Holm–Sidack test) concentrations in the organic layer; the values in the Corsican pine stand are higher than in that of Black locust for organic matter (OM) and for carbon (C), consistently with more productive vegetation that lead to higher stores of organic C (Trumbore and Torn, 2003). Nitrogen (N) concentration is higher in Black locust than in Corsican pine as found for trees capable to symbiotically fix nitrogen (Vitousek et al., 1987; Nagel and Griffin, 2001). No significant difference between the two stands was found for the mineral soil layers. However, as above reported organic matter content in mineral soil layers of Black locust, tend to be higher than in Corsican pine in spite of the higher amount of organic matter occurring in the organic layer of the latter. This confirm the results reported in Fig. 18 and strenghten the hypothesis concerning the role of the recalcitrance (Kononova, 1975; Schlesinger, 1977; Sequi, 1989; Trumbore, 1993) of the organic material derived from Black locust litter.

The C/N ratio, generally, decreases with depth in the organic and in the mineral soil layers.

In table 5 are shown concentrations of organic matter, carbon and nitrogen values in organic and mineral layers of the cronosequence. There are significant ($p < 0.05$, Dunn's test) differences between stands for concentrations of organic matter in the mineral layers, for carbon in both the organic and the mineral layers, and for nitrogen in the mineral layers. In particular, in the mineral layers, organic matter and carbon in the 96 year–old Stone pine stand are higher than in the younger Stone pine stands (66 and 36 year–old); this is in accordance with the trend described by Wardle et al. (1997).

Table 5. Concentrations (as percent of dry total soil mass) of Organic Matter (OM), Carbon (C) and Nitrogen (N) in organic layer and in mineral layer (at different depth) in three Stone pine stands on Mount Vesuvius. Values are means with standard error in parentheses. C/N is the ratio Carbon to Nitrogen.

	Stone pine											
	36 yrs old				**66 yrs old**				**96 yrs old**			
	OM	C	N	C/N	OM	C	N	C/N	OM	C	N	C/N
O. layer												
Litter n = 20	84.07 (± 5.53)	41.92 (± 1.96)	0.92 (± 0.03)	45.6	91.29 (± 3.56)	46.43 (± 0.39)	0.92 (± 0.03)	50.4	95.92 (± 0.37)	47.80 (± 0.42)	0.85 (± 0.03)	55.9
Humus n = 20	55.29 (± 6.38)	28.03 (± 2.71)	0.86 (± 0.07)	32.7	71.31 (± 4.07)	34.31 (± 1.86)	1.00 (± 0.04)	34.3	71.25 (± 2.20)	34.14 (± 1.37)	1.04 (± 0.04)	32.7
M. layer												
0-5 n = 20	2.14 (± 0.47)	0.74 (± 0.13)	0.12 (± 0.02)	6.3	3.68 (± 0.29)	1.52 (± 0.14)	0.27 (± 0.04)	5.7	9.81 (± 1.30)	5.17 (± 0.76)	0.34 (± 0.03)	15.2
5-10 n = 19-20	1.24 (± 0.18)	0.32 (± 0.05)	0.07 (± 0.02)	4.3	3.19 (± 0.26)	1.14 (± 0.09)	0.23 (± 0.03)	5.0	5.28 (± 0.61)	2.25 (± 0.29)	0.22 (± 0.02)	10.0
10-15 n = 18	1.03 (± 0.19)	0.31 (± 0.06)	0.06 (± 0.01)	5.5	3.34 (± 0.34)	1.22 (± 0.19)	0.26 (± 0.05)	4.7	4.27 (± 0.42)	1.44 (± 0.18)	0.21 (± 0.03)	6.8

n = number of samples.

In the organic layer, carbon concentration was higher in the 96 year–old Stone pine stand than in the youngest stand (36 year–old), consistently (Trumbore and Torn, 2003) with the higher litter production. Also nitrogen concentrations in the mineral layers differed (Batjes, 1996; Koch and Makeschin, 2004; Wardle et al., 1997). The values of the C/N ratio, generally decreases with depth in the organic and mineral layers. Along the cronosequence C/N ratio values in different mineral layers are higher ($p < 0.05$) in the older stand than in the younger stands.

5.4 NMR spectra and comparison of all five stands

From ^{13}C-CPMAS NMR spectra of newly shed litter, litter and humus layers (Figs. 19 and 20), following the profile downwards it is evident that the concentration of compounds considered more stable increase, from Alkylic C to Carboxylic C (Tables 6 and 7). The high amounts of paramagnetic compounds, that cause a broadening of spectral lines and reduce the signal-to-noise ratio, in humus of the 36 year–old Stone pine stand have decreased the sensitivity of solid–state ^{13}C-NMR, thus it is impossible to identify the four main regions (Wilsonet al., 1981; Baldock et al., 1987; Dai and Johnsonn, 1999; Evans et al., 2001).

The ratio between the values for the alkylic C region (A) and the O–alkylic one (OA) (Fig. 21) indicates the degree of decomposition of plant residues in soil (Baldock et al., 1997). Generally we can notice an increase in values for A/OA from newly shed litter to humus, that suggests an increasing degree of decomposition in that order (Amalfitano et al., 1995; Baldock and Preston, 1995; Beyer et al., 1993). Another index to evaluate decay degree of OM is the percentage of aromaticity (%Ar) (Fig. 22) and we can notice an evident trend increasing from newly shed litter to humus, with higher values for soil in the Black locust stand than in the other ones.

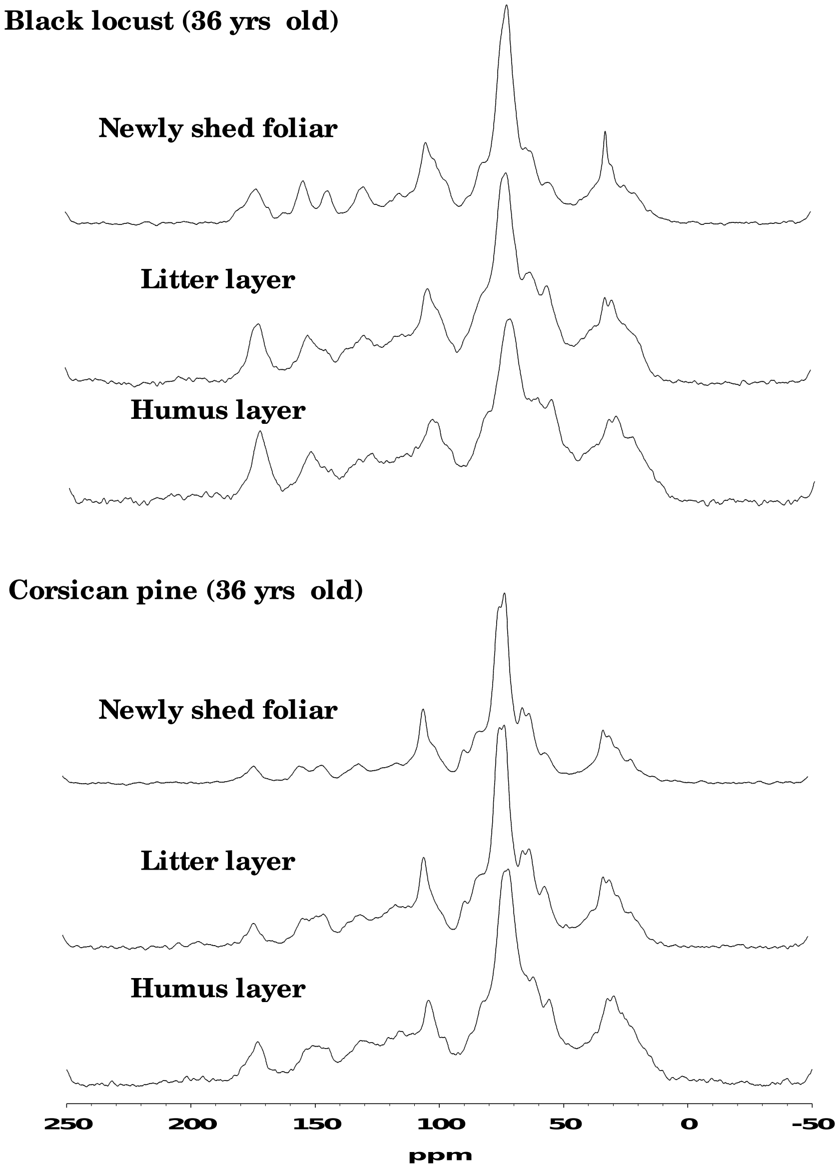


Figure 19. ^{13}C-NMR spectra of organic matter of newly shed foliar, as well as of the litter and humus layers in Black locust and Corsican pine stands on Mount Vesuvius.

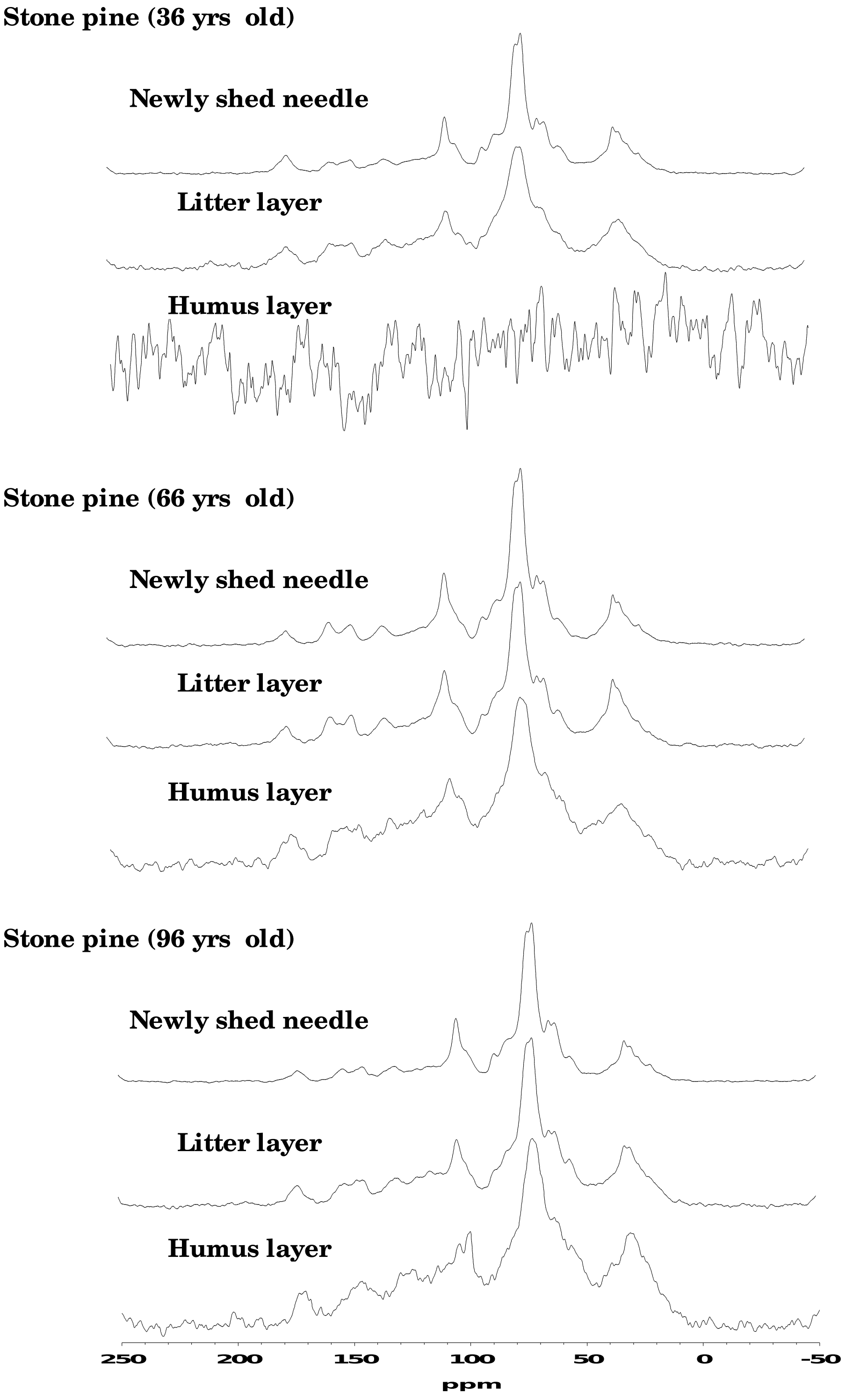


Figure 20. ^{13}C NMR of organic matter of newly shed needle litter as well as of the litter and humus layers in a Stone pine cronosequence on Mount Vesuvius .

Table 6. Atrio del Cavallo on Mount Vesuvius. Black locust and Corsican pine stands: peak integrations of four main regions of ^{13}C CPMAS NMR spectra for newly shed litter as well as litter and humus layers. A/OA: ratio of the value in the alkyl carbon region to those of the O-alkyl region; % Ar: aromaticity degree. “newly shed” are leaves/needles picked up after one day; “litter” and “humus” are organic layer over the top of mineral soil.

	ppm	newly shed	litter	humus
Black locust (36 yrs old)				
Carboxylic C	165 – 190	6	7	8
Aromatic C	110 – 165	17	19	19
O – Alkyl C	45 – 110	59	56	53
Alkyl C	0 – 45	18	18	20
A/OA		0.31	0.33	0.39
% Ar		7.41	8.10	9.54
Corsican pine (36 yrs old)				
Carboxylic C	165 – 190	4	3	6
Aromatic C	110 – 165	14	19	20
O – Alkyl C	45 – 110	65	61	54
Alkyl C	0 – 45	17	17	21
A/OA		0.25	0.28	0.39
% Ar		4.40	4.00	7.08

Table 7. Stone pine stands along a cronosequence on Mount Vesuvius: peak integrations of four main regions of ^{13}C CPMAS NMR spectra for newly shed litter as well as litter and humus layers. A/OA: ratio of the value in the alkyl carbon region to those of the O-alkyl region; % Ar: aromaticity degree. “newly shed” are needles picked up after one day. “litter” and “humus” are organic layer over the top of mineral soil.

	ppm	newly shed	litter	humus
Stone pine 36 yrs old				
Carboxylic C	165 – 190	5	6	nd
Aromatic C	110 – 165	14	21	nd
O – Alkyl C	45 – 110	63	55	nd
Alkyl C	0 – 45	19	18	nd
A/OA		0.30	0.33	nd
% Ar		5.46	6.98	nd
Stone pine 66 yrs old				
Carboxylic C	165 – 190	4	4	4
Aromatic C	110 – 165	15	19	21
O – Alkyl C	45 – 110	65	59	57
Alkyl C	0 – 45	16	18	17
A/OA		0.25	0.31	0.31
% Ar		4.28	5.07	5.13
Stone pine 96 yrs old				
Carboxylic C	165 – 190	3	4	4
Aromatic C	110 – 165	14	19	21
O – Alkyl C	45 – 110	67	57	54
Alkyl C	0 – 45	16	19	21
A/OA		0.24	0.33	0.39
% Ar		3.05	5.12	5.58

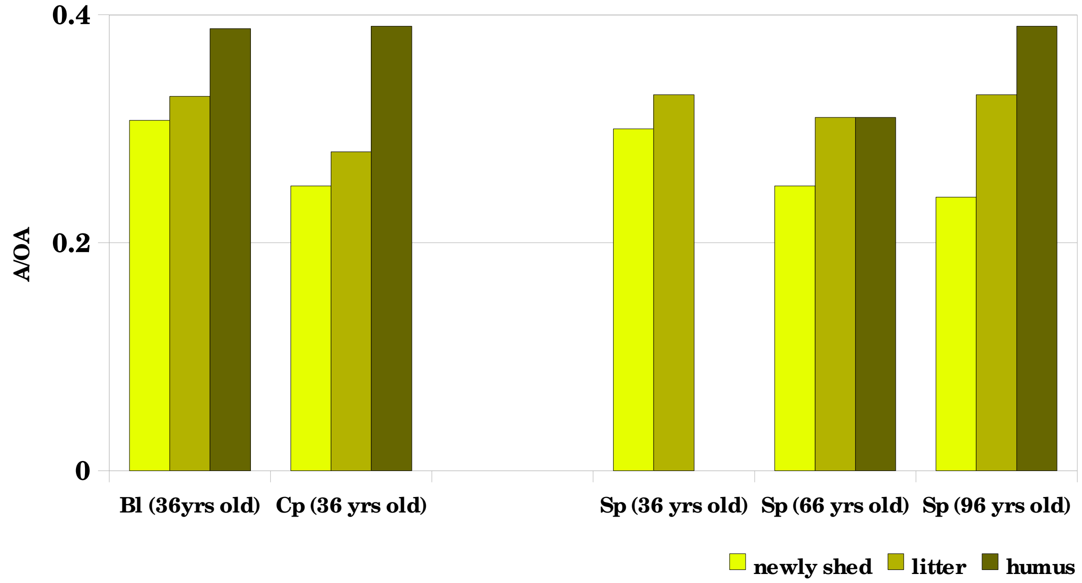


Figure 21. Ratio between alkyl carbon (A) and O–alkyl carbon (OA) obtained with ^{13}C-CPMAS NMR technique. Bl = Black locust; Cp = Corsican pine; Sp = Stone pine on Mount Vesuvius.

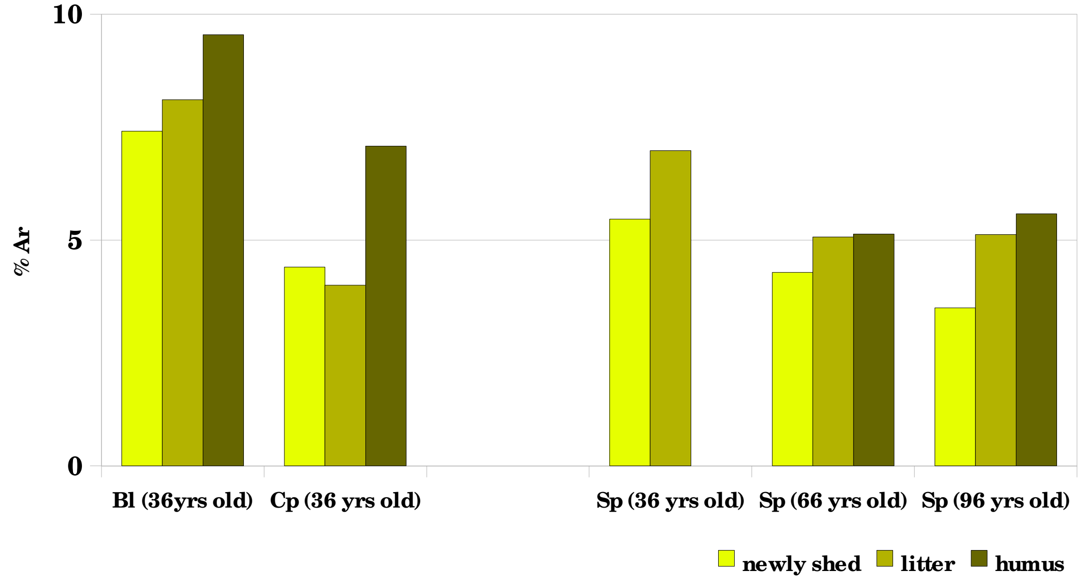


Figure 22. Percentage of aromatic compounds (% Ar) obtained with ^{13}C CPMAS NMR technique on samples of Bl = Black locust; Cp = Corsican pine; Sp = Stone pine on Mount Vesuvius.

In table 8 are shown amounts (g/m^2) of Soil Organic Carbon (SOC) in organic and mineral layers of all five stands.

Table 8. Amounts of Soil Organic Carbon (SOC) in organic and in mineral layers (at different depth) of stands on Mount Vesuvius. Values are means with standard error in parentheses.

	Black locust	**Corsican pine**		**Stone Pine**	
	36 yrs old		**36 yrs old**	**66 yrs old**	**96 yrs old**
			g/m^2		
Organic layer					
Litter n = 15-20	848.32 (± 59.91)	1403.16 (± 150.97)	475.77 (± 49.27)	1293.19 (± 302.24)	1446.31 (± 247.92)
Humus n = 9-16	788.06 (± 76.20)	1298.73 (± 229.65)	441.66 (± 81.35)	939.60 (± 207.20)	1291.12 (± 251.30)
SUB TOTAL	***1636.38***	***2701.89***	***917.43***	***2232.79***	***2737.43***
Mineral layer					
0-5 cm n = 17-20	98.17 (± 10.74)	73.45 (± 7.98)	56.79 (± 4.02)	114.08 (± 7.80)	144.98 (± 32.12)
5-10 cm n = 17-20	25.94 (± 3.86)	28.63 (± 3.88)	44.05 (± 4.92)	94.51 (± 5.38)	104.50 (± 9.66)
10-15 cm n = 11-18	12.24 (± 2.00)	14.45 (± 1.56)	35.72 (± 5.10)	82.21 (± 6.38)	78.34 (± 10.97)
SUB TOTAL	***136.35***	***116.53***	***136.56***	***290.80***	***327.82***
TOTAL	**1772.74**	**2818.43**	**1053.99**	**2523.59**	**3065.25**

n = number of samples.

There are significant ($p < 0.05$, Dunn's test) differences in the Stone pine cronosequence for amount of soil organic carbon, in both the organic and the mineral soil layers, following the series: 36 year–old stand < 66 years old < 96 years old stand; this is in agreement with the trend described by Wardle et al. (1997) and Bauhus et al. (1998). For the two adjacents stands (Corsican pine vs

Black locust) the differences were significant only for the organic layer with values higher in Corsican pine soil than in Black locust, consistently with more productive vegetation that lead to higher stores of organic C (Trumbore and Torn, 2003).

Comparing all stands, the 36 year–old Stone pine stand had lower amounts of carbon in the organic layer than the other stands. In the mineral soil layers the 66 and 96 year–old Stone pine stands had higher values than the stands of Black locust, Corsican pine and the 36 year–old stand of Stone pine, in according to decrease in C assimilation efficiency with stand age as found by Bauhus et al. (1998).

The amounts (g/m^2) of organic matter and carbon reported in Fig. 23 show how much C may accumulate during 36–96 years in the soil of M. Vesuvius forests on young volcanic substrate and the differences among three diverse plant cover (Gerighausen, 2002; Koch and Makeschin, 2004).

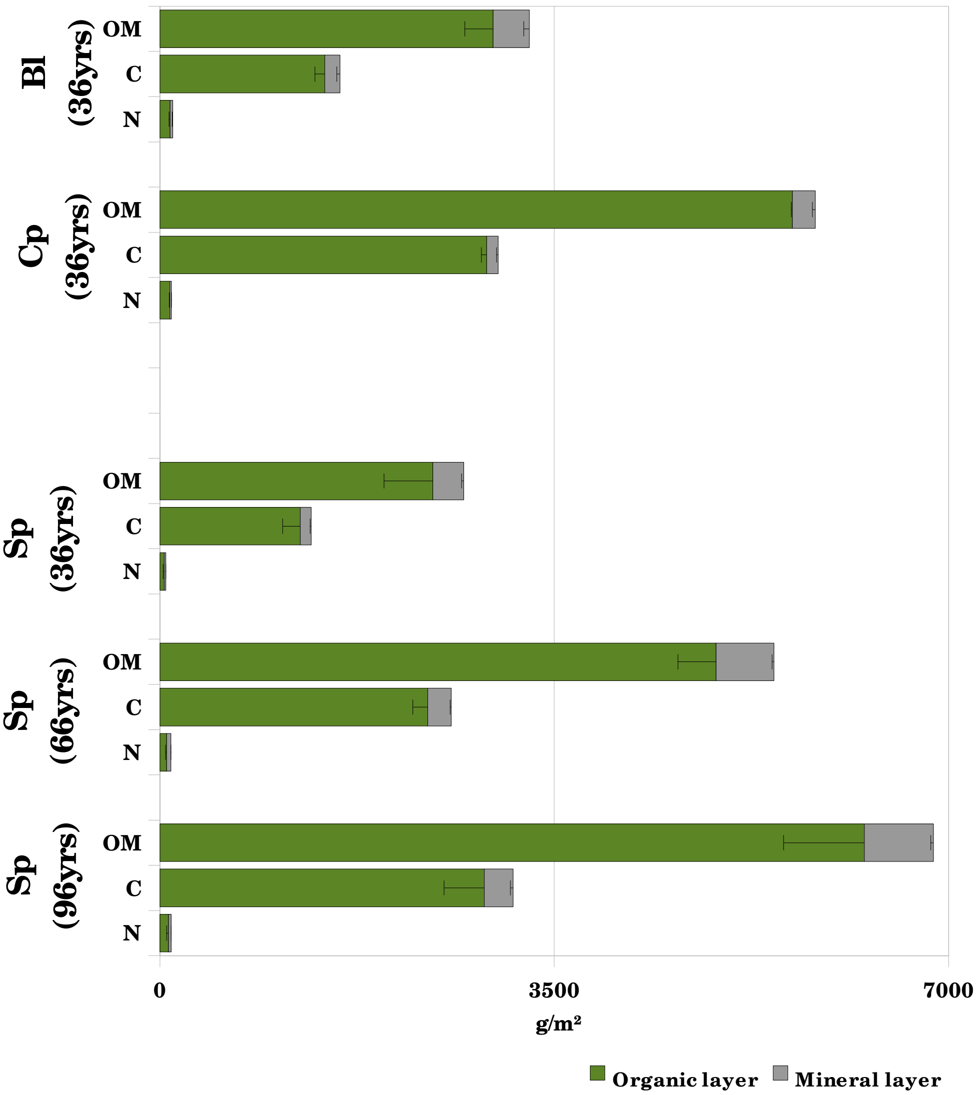


Figure 23. Amounts of Organic Matter (OM), Carbon (C) and Nitrogen (N) in organic (litter and humus) and mineral layers (0 – 15 cm depth) of five stands on Mount Vusuvius. Bl = Black locust; Cp = Corsican pine; St = Stone pine.

5.5 Allelopathic effects of Black locust litter on soil microbial activity

In the Black locust stand, as compared to that of Corsican pine the amount of soil organic matter (0–5 cm) was higher (Tab. 4 and Fig. 18) whereas soil microbial biomass and activity were lower (Fig. 24).

With two way analysis of variance, statistically significant differences ($p < 0.05$) have been found between Corsican pine and Black locust soils for soil microbial biomass (mgC/gC_{org}=135.4 vs 45.6), basal respiration ($mgCO_2/gC_{org}$=2.3 vs 0.6) and carbon endogenous mineralization (CEM) ($mgC\text{-}CO_2/gC_{org}$= 0.63 vs 0.16), a parameter that express the efficiency of microflora to mineralize organic carbon.

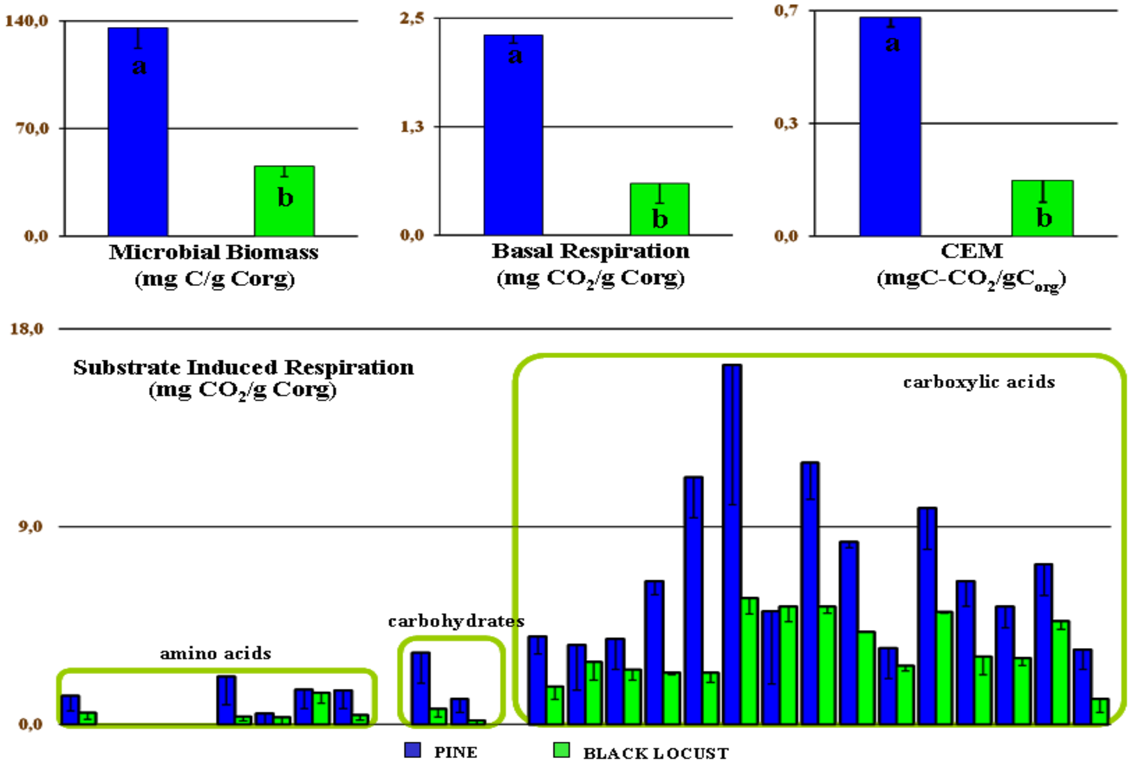


Figure 24. Biological parameters. Soil Microbial Biomass, Basal Respiration, CEM (= mineralization of organic carbon) and catabolic profile of microorganisms belong to Corsican pine (blue) and Black locust (green) soils (0-5cm).

Moreover statistically significant differences ($p < 0.05$) have been found between respiration rates from Corsican pine and Black locust soils after addition of 25 simple organic substrates (Fig. 24).

The data suggest that carbon tends to be accumulated in the soil under Black locust as compared to the coeval Corsican pine forest implanted on the same substrate (Vittozzi et al., 2007) (see Tab. 4).

It is know that Black locust leaves contain allelopathic compounds that reduce the growth of herbaceuous species (Nasir et al., 2005; Fujii et al., 2006), that phytotoxicity occur in decomposing plant litter (e.g. Shaukat et al., 2004; Bonanomi et al., 2006), and that allelopathic compounds are released during organic matter decomposition (van der Putten et al., 1997; Blum et al., 1999; Armstrong and Armstrong, 2001). Moreover Colleagues of Chemistry and Biochemistry Department have isolated from extracts of Black locust litter, by silica gel column chromatography, and identified by ^{1}H NMR spectra, 4–hydroxyacetophenon, a substance known to have inhibitory effects on eight plant species (Kalinova et al., 2007) and to cause a 53–67% decrease in ethanol production in *Saccharomyces cerevisiae* (Klinke et al., 2003). Hence I hypothesize that soil microbial activity might be inhibited by the presence of allelopathic substances in Black locust litter.

Although allelopathic effects of humus phenolics on growth and respiration of mycorrhizal fungi have been described (Souto et al., 2000a), there is a lack of knowledge about allelopathic effects of decomposing litter on soil microbial populations and activity.

In laboratory studies I obtained statistically significant differences ($p < 0.05$) in Corsican pine soil between soil samples amended with Black locust litter extracts and control soil treated with distilled water for basal respiration rates (Fig. 25), microbial biomass and carbon endogenous mineralization (Tab. 9). No significant difference has been found in Black locust soil between amended and control soil for the same parameters.

Table 9. Biological parameters of Corsican pine soils, obtained after added of aqueous extracts of Black locust litter: DL (=decomposed litter), FL (=fresh litter) in laboratory studies. Values are means with standard error in parentheses.

		Range of dilutions (0.001%-100%)	
	Control	**DL**	**FL**
Basal respiration **(mg CO_2/gC_{org})**	3.2 (±0.2)	1.5-3.4 (±0.1)	3.3-2.2 (±0.2)
Microbial biomass **(mg C/gC_{org})**	239 (±5.24)	240-192 (±9.82-4.48)	286-215 (±8.77-11.18)
Carbon endogenous mineralization **(mgC-CO_2/gC_{org})**	0.88 (±0.05)	0.41-0.93 (±0.02-0.04)	0.91-0.59 (±0.05-0.08)

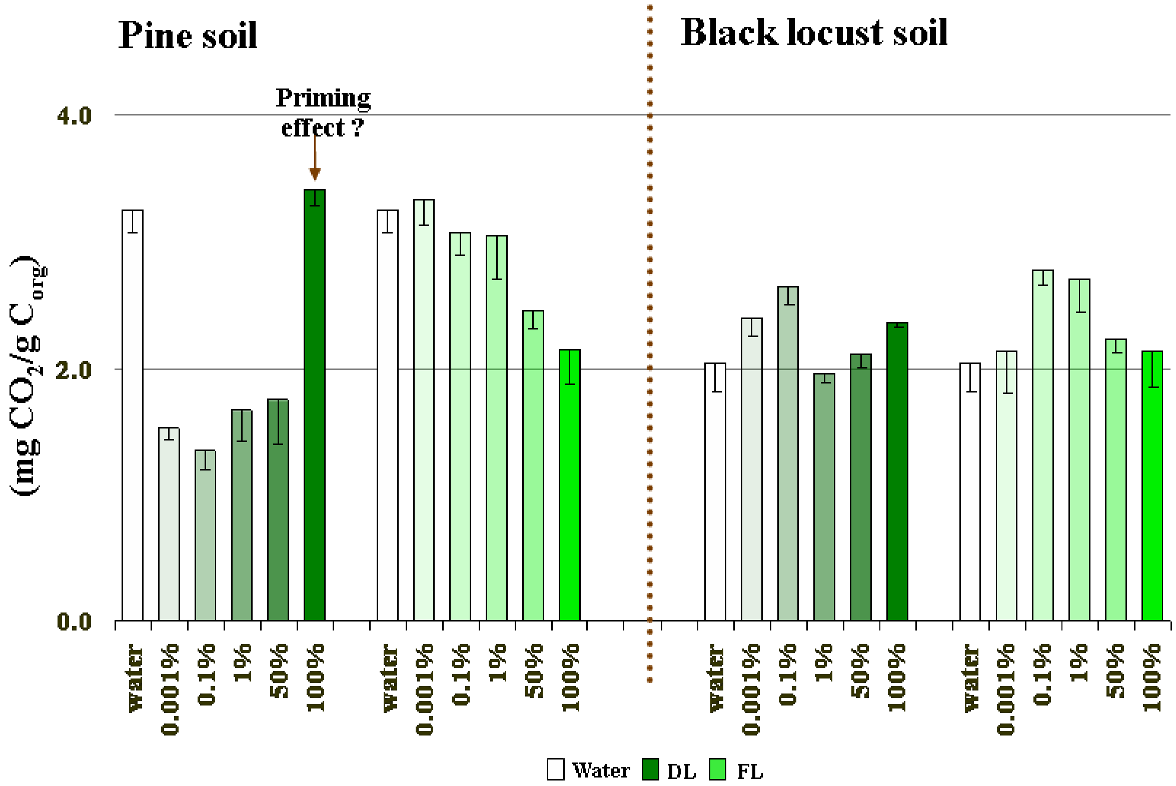


Figure 25. Basal respiration as related to extract dilutions of laboratory experiments.

The high response of Corsican pine soil amended with 100% extract from far decomposed Black locust litter (Fig. 25), might be explained by the occurrence of easily assimilable compounds in the most concentrated extract supplying energy for many dormant microorganisms (priming effect) (Kuzyakov et al., 2000). Similar results have been obtained for microbial biomass and endogenous mineralization coefficient.

The results of laboratory experiments suggest that the effects of aqueous extracts of far decomposed litter do not decrease significantly with dilution; in contrast the effects of aqueous extracts of fresh litter decrease significantly with dilution ($p < 0.05$). Aqueous extracts of Black locust litter suppress activity of microbial community of Corsican pine soil but have no effect on microbial community of Black locust soil (adapted to the presence of allelopathic compounds).

The results of field experiments are consistent with the result of laboratory experiments. Statistically significant differences ($p < 0.05$) have been found for microbial biomass between control soil and the soils under the two type of Black locust litter (Tab. 10 and Fig 26). Moreover the values of the microbial metabolic quotient, commonly considered a bio–indicator of disturbance, evidence that microbial processes in Corsican pine soil under Black locust litter are under a stress or disturbance regime, likely determined by the introduction of the alien litter. Active and total fungal mycelium seem to be repressed only by fresh litter extracts of Black locust (Tab. 10 and Fig. 26).

Table 10. Biological parameters of Corsican pine soils, obtained after added of Black locust litter: FL (=fresh litter) DL (=decomposed litter) in field studies. Values are means with standard error in parentheses.

	Control	**DL**	**FL**
Basal respiration **(mg CO_2/gC_{org})**	1.28 (±0.09)	1.29 (±0.20)	1.19 (±0.14)
Microbial biomass **(mg C/gC_{org})**	87.60 (±4.66)	80.08 (±10.90)	63.51 (±4.22)
Active fungal biomass **(mg bf/gC_{org})**	5.36 (±0.67)	6.66 (±0.95)	4.08 (±0.49)
Total fungal biomass **(mg bf/gC_{org})**	9.85 (±1.12)	10.38 (±1.36)	7.02 (±0.72)
Microbial metabolic quotient (qCO_2) **(μgC-CO_2/gC_{mic})**	4.44 (±0.47)	4.68 (±0.45)	5.179 (±0.28)

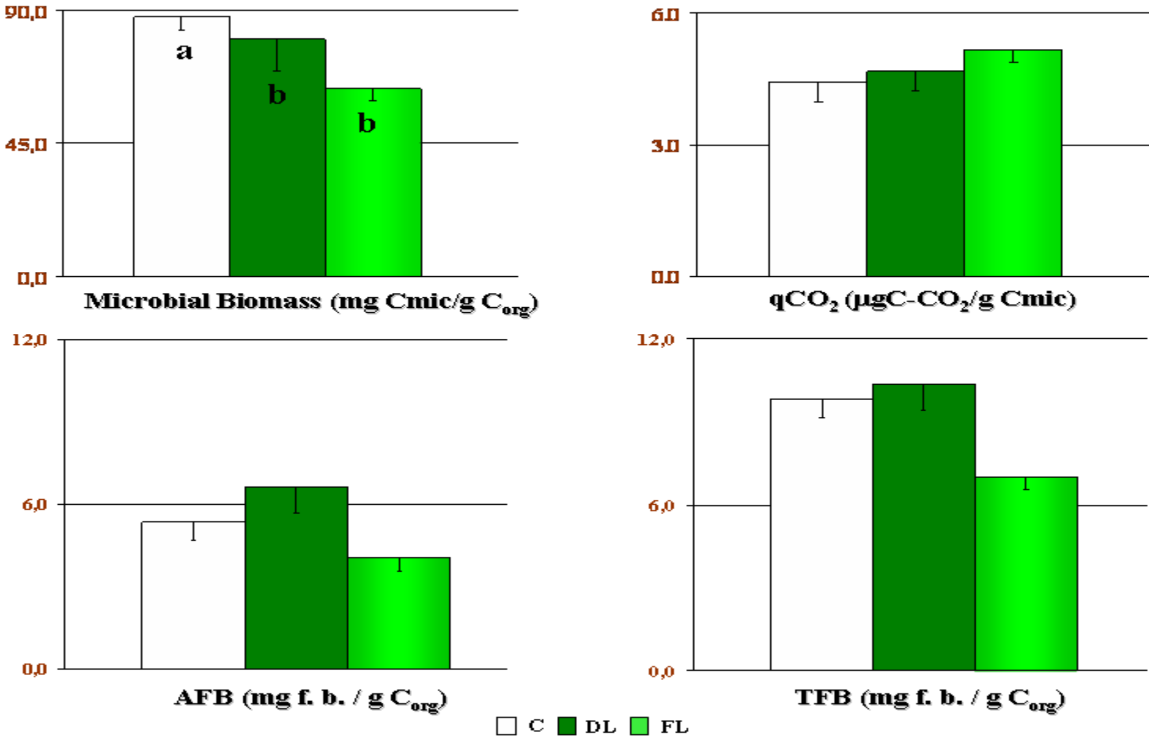


Figure 26. Biological parameters of field experiments. AFB = active fungal biomass, TFB = total fungal biomass. C = control soil, DL = soil under decomposed Black locust litter, FL = soil under fresh litter of Black locust.

The substrate induced respiration evidences statistically significant differences ($p < 0.05$) between control soil and soil under Black locust litter either fresh (FL) or decomposed (DL) (Fig 27).

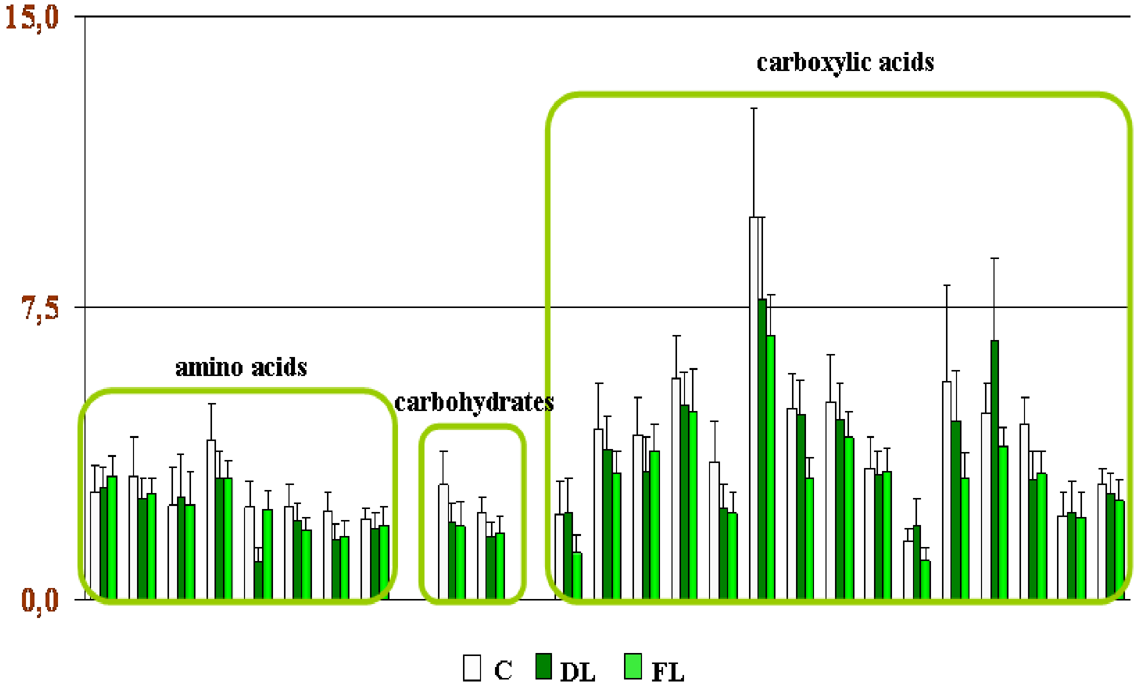
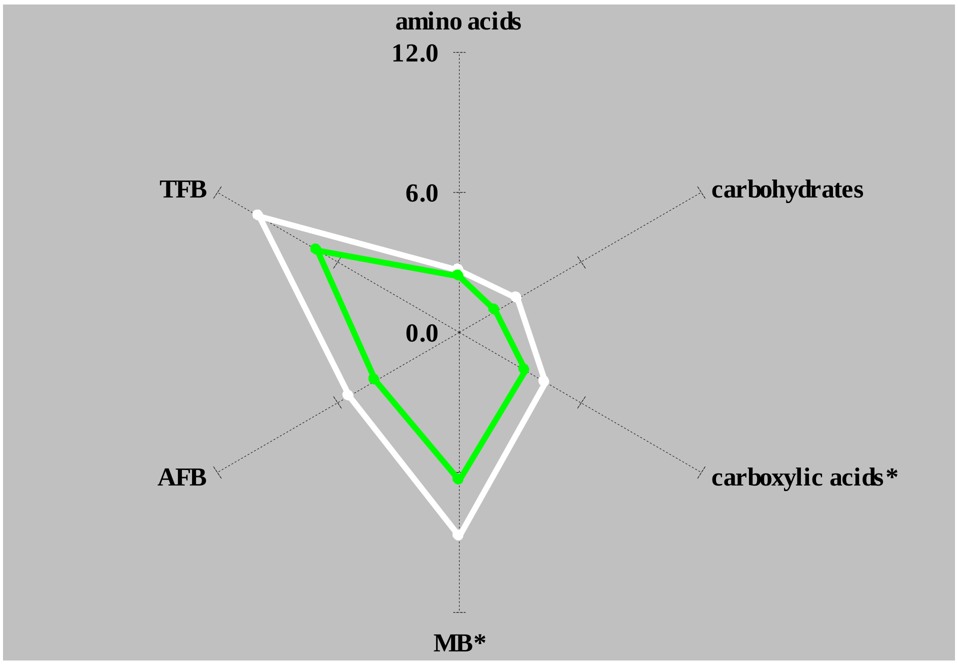


Figure 27. Substrate Induced Respiration (mg CO_2/g Corg), of soil microorganisms in field experiments, after addition of 25 different solutions. C = control soil, DL = soil under decomposed Black locust litter, FL = soil under fresh litter of Black locust.

The below radar plot with the main investigated parameters summarizes the results (Fig. 28): microbial activity of Corsican pine soil covered by Black locust litter is repressed (although the differences are not always statistically significant). Fresh litter (FL) seems to be more active than decomposed litter (DL) ($p < 0.05$).



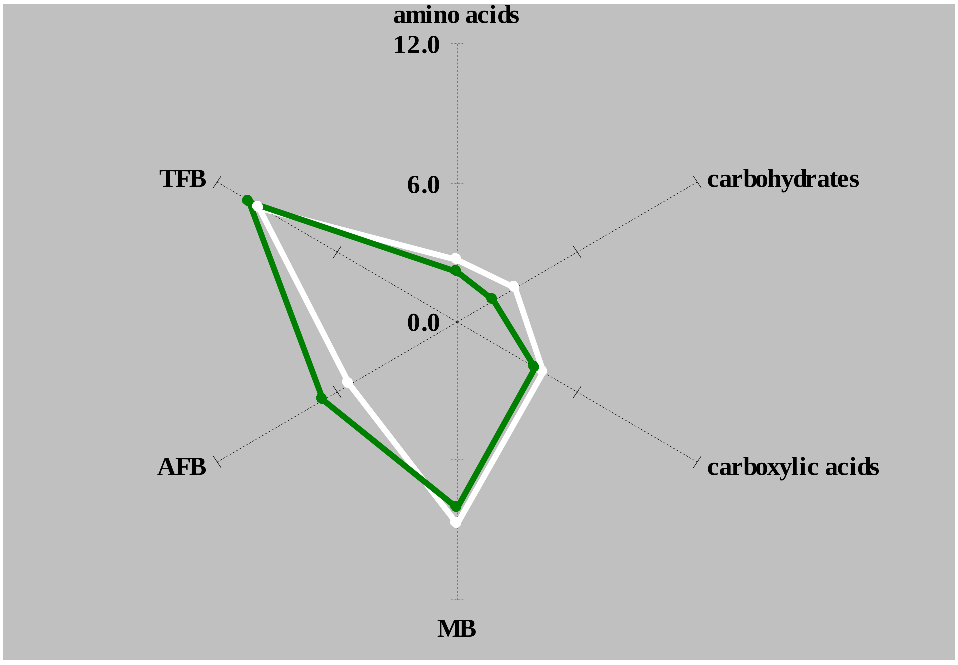


Figure 28. Radar plot. AFB = active fungal biomass, TFB = total fungal biomass, MB = microbial biomass. White line = control, bright green line = fresh Black locust litter, dark green line = decomposed Black locust litter. The asterisk means that only for marked parameters the differences are significant ($p < 0.05$).

6. CONCLUSIONS

The following conclusions may be drawn from the data presented:

1. In the two coeval (36 yrs old) stands on lapillus of the last eruption of Vesuvius, with a different tree cover (Black locust and Corsican pine), litter fall (years 2006–2008) is higher in the Corsican pine than in the Black locust stand ($P < 0.05$) even if Blackberry brambles (spreaded understory of Black locust stand) litter is added to that from Black locust trees. Tree basal area is similar in the two stands, however the number of stems in the Corsican pine stand is 350 vs 2091 stems; the Total Litter Fall over Basal area (TLF/BA) is 90.7 Kg/m^2 for Black locust vs 210.2 Kg/m^2 for Corsican pine and the higher litter fall amount of Corsican pine corresponds to the higher biomass.

   In the organic soil layers amounts of organic C are higher ($P < 0.05$) in Corsican pine as compared to Black locust stand (g/m^2 = 2701.9 vs 1636.4), while in the mineral layers organic C amounts are lightly higher (but not statistically different) in Black locust than in Corsican pine soil (136 vs 116 g/m^2). SOM amounts determined along the 15 cm depth profile, in each one-cm layer, also show an unexpected, significantly ($p < 0.05$)

higher accumulation of C in the deciduous stand than in the coniferous one (4.87 vs 1.59%).

NMR data show that the aromaticity degree of humus is higher for Black locust than for Corsican pine (% Ar = 9.54 vs 7.08) thus suggesting that Black locust organic residues in the late stage of decomposition are more recalcitrant as compared to Corsican pine residues.

A further interesting result is that, in spite of larger amounts of soil organic matter, smaller values of soil microbial activity have been measured in the mineral soil under Black locust than in the Corsican pine stand. As the possibility that microorganisms activity could be inhibited by high nitrogen content in Black locust stand has to be excluded (N concentrations are not significantly different between Corsican pine and Black locust), the allelopathic effect of Black locust litter on soil metabolism has been tested. The results allow to conclude that Black locust litter: i) inhibit soil microorganisms activity, ii) produces a recalcitrant residue, rich of aromatic compounds, including 4–hydroxyacetophenon, known for its allelopathic potential. This may lead to high accumulation of organic matter in the soil and thus to high C sequestration.

2. In the Stone pine cronosequence, litter fall (2006–2008) increases gradually from the younger stand (36 yrs old) to the oldest one (96 yrs old) and is positively related to tree basal area (BA); Total Litter Fall over Basal Area (TLF/BA) also increases with stand age (123 to 145 Kg/m^2). Moreover chemical analyses of organic and mineral soil layers clearly indicate the accumulation of C with stand age (g/m^2= 1053.9 for 36 yrs old stand vs 2523.5 for 66 yrs old one and vs 3065.2 for 96 yrs old one).
3. The comparison of all pine stands shows that the trend of litter fall and C sequestration, increasing from younger to older stand, doesn't hold if the Corsican pine stand is included; indeed the 36 yrs old Corsican pine stand is more similar to the 66 yrs old Stone pine stand than to the even aged one. It is not possible at now to indicate the reasons for this: many factors that have not been analysed in details in the current research, such as biological (higher production capacity of Corsican pine?), and site characteristics (local climate, litology and soil, slope) could be responsible for the observed differences.

It is important to point out that the increase of C sequestration with stand age has been likely underestimated given that the deep soil profile of the oldest Stone pine stand has been analysed for SOM content only up to 15 cm depth.

Moreover it would be more appropriate: i) to determine litter-fall over a longer time span to investigate the influence of climatic variability, ii) try to correlate litter production to climatic patterns at the different stands, iii) to compare C sequestration also along a chronosequence of Corsican pine.

Lastly the reported NMR data refer only to the organic soil layers. Due to the presence of paramagnetic compounds into the mineral layers, the SOM spectra were hill defined. It is necessary to improve the extraction technique to obtain suitable spectra allowing to characterize the properties of SOM in the mineral layers.

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