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C and N Pools In Afforested Pine Forests and Natural Shrublands

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Research Article

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Abstract

Aims

Plant cover and microclimatic conditions can profoundly alter the balance between productivity and decay, with relevant effects on soil C and N pools. In this contest, the aim of the present study was to assess how, in Mediterranean areas, soil properties and C and N sequestration differs between afforested pine forests and natural shrublands in different microclimatic conditions at low and high elevations.

Methods

The study was performed along the soil profile distinguishing between the organic layers, fermentation and humic layers, and surface mineral soils. The comparison between plant covers and elevations were carried out for C and N pools and soluble fractions, molecular characterization by solid state ¹³C NMR of organic layers and by ¹HNMR of soil soluble fractions, potential mineralization rates and microbial and fungal amounts.

Results

Our data confirm that coniferous tree species sequester C faster than shrubs and herbaceous species especially at low elevation under favourable microclimatic conditions. Soil C and N pools reflect changes in the chemical composition of the upper organic layers and of soil soluble organic matter. In pine forests, the higher concentration of N in the upper organic layer speeds up the N loss in the fermentative layer and stimulates humus formation and C accumulation at low elevations.

Conclusions

Plant cover and microclimatic conditions drive the C sequestration rate and the soil organic matter stability. Chemical changes highlighted by nuclear magnetic resonance spectroscopy can clarify patterns of decay processes and help to make predictions in a climate change scenario.

Introduction

Forests cover approximately 30% of the Earth's surface and are responsible for global carbon (C) cycle regulation and C sinks, as they store approximately 80% of all aboveground terrestrial C and 40% of soil C (Goodale et al. 2002; Lal 2004; FAO 2012).

Soil C sequestration largely depends on the net input of dead organic matter and the release of CO₂ due to decomposition (Berg and McClaugherty 2020). Plant cover affects C sequestration rates mainly through litterfall and the size of the stable litter fraction formed during decomposition (Berg 2018). Generally, conifer litter decomposes more slowly than deciduous litter, and hence, there is a greater build-up of conifer litter and greater soil C accumulation (De Marco et al. 2013; De Marco et al. 2018; Silver et al. 2004). Afforestation, for example, may increase C and nitrogen (N) stocks due to the input of

aboveground and belowground tree litter (Egli et al. 2008; De Marco et al. 2013; Lilienfein et al. 2003), and in afforested areas, C and N contents increase with time, which results in substantial C storage (De Marco et al. 2021; Innangi et al. 2017). In addition, C and N sequestration are strongly influenced by microclimatic conditions (Kobler et al. 2019). In fact, soil C and N sequestration typically increases with elevation due to the slower soil organic matter decomposition caused by lower temperatures (Schindlbacher et al. 2010; Tashi et al. 2016). However, an increase in net primary production (NPP) as well as in soil CO₂ efflux (Swetnam et al. 2017), a fast litter mineralization and an increase in soil organic matter stability (Berg 2018) can be expected with decreasing forest elevation. In addition, litter chemical composition varies with climate, and the litters richest in nutrients, mainly N, are generally correlated with high mean annual temperatures (Berg and McClaugherty 2020). A high litter N concentration can indeed induce fast decay in the early decomposition stage but may delay the degradation of lignified tissue in the late decomposition stage, potentially promoting the stabilization mechanisms of soil organic matter (Hagedorn et al. 2003; Pregitzer et al. 2008). Thus, soil C sequestration depends on a combination of mineralization and humification processes related to changes in the chemical composition of organic detritus and in the stability of the soil organic residue. Although a decrease in alkyl C and an increase in aliphatic C were observed during humification, regardless of plant species (Ono et al. 2011; Osono et al. 2008), many factors, such as chemical litter quality and microclimatic conditions, affect soil C and N cycles (Yan et al. 2018), and further investigation is needed.

Mediterranean environments, characterized by wide annual climatic variations and high plant biodiversity, are particularly interested but have received little research from this perspective (Roces-Diaz et al. 2021). Notwithstanding, these environments have lower productivity and a lower C sink than other Northern or Central European ecosystems (Pan et al. 2011). Moreover, climate change, with annual increases in temperature and decreases in precipitation, could drastically affect C sequestration in Mediterranean forests (Lindner and Calama 2013).

In this context, the aim of the present study was to assess the role of afforested pine forests and natural shrublands on soil C and N sequestration in a Mediterranean area. In addition, given the importance of microclimatic conditions for soil processes in the Mediterranean region, the study was performed at different elevations (600 m and 900 m a.s.l.). To reach this aim, comparisons between soils under different plant covers and at two elevations were carried out for the C and N pools and soluble fractions in organic and mineral soil layers, the soil organic carbon quality through molecular characterization by solid-state ¹³C NMR of organic layers and ¹H NMR of soil soluble fractions, and the soil organic matter turnover in the upper mineral soil through measurements of potential mineralization rates and microbial and fungal amounts.

Materials And Methods

Study area

The study was located inside Vesuvius National Park (Naples, southern Italy). Mount Vesuvius is a volcano located on the southern margin of the Campania Plain (southern Italy), and it has been quiescent since 1944 after 3 centuries of activity with 49 documented eruptions. The slopes of Mount Vesuvius are a complex mosaic of vegetation dominated by herbs and shrubs that naturally evolve towards oak forests. Particularly abundant are Spanish broom (*Spartium junceum* L.), black locust (*Robinia pseudoacacia* L.), tree of heaven (*Ailanthus altissima* Mill.), Mount Etna broom (*Genista aetnensis* DC) and young holm oaks. In the past century, the slopes of Mount Vesuvius were afforested to stabilize unstable materials derived from the last eruptions to prevent landslides that threaten the areas at the bottom of the mountain. To achieve this goal, black pine (*Pinus nigra* L.), maritime pine (*Pinus pinaster* Aiton) and stone pine (*Pinus pinea* L.), which grow naturally throughout the Mediterranean region, were employed for afforestation (di Fusco, 2000). In particular, stone pine is the most commonly used afforestation species because it can grow in low-nutrient and highly drained unconsolidated soils. Additionally, its large heavy seeds have abundant reserves that allow rapid root development and enable it to start consolidating the soil within a short time span.

According to USDA soil taxonomy, the studied soil is classified as Lepty-vitric andosol (di Gennaro et al. 2002), originating from volcanic parent material of the Vesuvius eruptions from 1891 to 1944. It should be emphasized that the chemical composition of the leucititic tephrites-phonolites deposited by the eruptions has been the same over the last two centuries (Santacroce 1987; Santacroce and Sbrana 2003).

The climate conditions of the study area are typically Mediterranean and characterized by summers with frequent drought and rainy autumns and winters. The mean annual temperature is 13.6°C, whereas the annual precipitation reaches 961 mm (Osservatorio vesuviano – 605 m a.s.l.; 40°49'N; 14°24'E).

Sampling along the soil profile

The sampling along the soil profile was performed in May 2017 at sixteen sites: eight covered by stone pine (named pine forest, P) and eight covered by shrub (named shrubland, S) and herbaceous species. Samplings of organic and mineral soil layers in pine forests and in shrublands were performed at 600 m a.s.l. (low elevation-L) and 900 m a.s.l. (high elevation-H).

At each site, the organic horizon above the mineral soil was sampled at six points using a square sampling frame (20×20 cm), and all organic horizon, distinguishing among the organic layers (OL) and fermentation and humic layers (OF + OH), was collected. The litter from the OL layer, consisting of plant residues easily discernible by the naked eye, was manually separated from strongly fragmented and amorphous material belonging to the OF + OH layer (De Marco et al. 2016). In addition, at the same site, six subsamples of the surface mineral layer (0-10 cm) were collected and mixed together to obtain a homogeneous sample to perform the analyses.

Organic horizon analyses

In the laboratory, the samples of organic layers and fermentation and humic layers were weighed, and the results were expressed as g m⁻². In addition, the samples were analysed to determine the total C and N concentrations, which were evaluated using samples that were oven-dried (75°C, until constant weight) and ground (Fritsch Analysette Spartan 3 Pulverisette 0) and then analysed by gas chromatography (Thermo Finnigan, CNS Analyzer).

In addition, samples were analysed for 13C CPMAS NMR spectra using a Bruker AV300 Spectrometer Solid-state 13C Nuclear Magnetic Resonance (NMR) with cross polarization and magic angle spinning (CP/MAS). The 13C CPMAS spectra showed peaks in the whole range of chemical shifts between 0 and 200 ppm, with overlapping peaks due to a great diversity of compounds in the samples. The spectra were divided into six chemical shift regions representative of the major types of carbon present in the litter (carboxyl-C: 200 – 160 ppm; phenol-C: 160 – 145 ppm; aryl-C: 145 – 110 ppm; O-alkyl-C: 110 – 60 ppm; methoxyl-C: 60 – 45 ppm, and alkyl-C: 0–45 ppm). Carbon bond types have been identified in previous reference studies (Mathers et al. 2007). The mass of each C fraction per gram of organic material was calculated from peak integrations of NMR spectra and the total C per gram.

Mineral soil analyses

Chemical analyses

In the laboratory, the soil samples were sieved (2 mm) and divided into different portions to measure, in triplicate, the following parameters: pH, water (WC) and organic matter (OM) contents, and total and soluble C and N contents. The pH was measured in a soil: distilled water (1:2.5 = v:v) suspension by an electrometric method (Colombo and Miano 2015); the WC was determined by drying fresh soil at 105°C until a constant weight was reached; OM content was obtained by multiplying 1.724 (Pribyl 2010) for the organic carbon content, evaluated by gas-chromatography (Thermo Finnigan, CNS Analyzer) in samples previously treated with HCl (10%). The total C and N contents were evaluated as previously described for the organic layer samples. The mineral soil samples of all stands contained considerable amounts of mineral and paramagnetic compounds (Memoli et al. 2018), which decreased the sensitivity of solid–state ¹³C NMR, giving poor resolution spectra and low signal–to–noise ratios; therefore, these data were not considered.

Water soluble organic matter was determined according to Garcia et al. (1991 modified). Oven-dried (105°C) samples were soaked in 200 mL of distilled water and stirred for 24 h (Universal Table-Shaker 709, 130 rpm) with two sonications. The amount of water-soluble organic matter was obtained by the difference between the dry weight (75°C) of the sample before and after soaking. The water extracts were centrifuged at 5000 rpm for 15 min and filtered (Whatman Ø 15 μ m). The concentrations of soluble C and N were evaluated by gas chromatography (CNS analyser—Thermo Finnigan, Flash 112 Series EA) after lyophilization of the water extracts.

The chemical composition of the water extracts was assessed by the 1H NMR spectra using a Bruker AV 400 Spectrometer Liquid-state (frequency: 400.13 MHz; pulse delay: 1.0 s; acquisition time: 278 s; line

broadening factor: 1 Hz). The spectra were divided into five chemical shift regions representative of the major types of protons present in the extracts (Silverstein et al. 2015): carboxylic acids: 12 – 8 ppm; aromatic compounds: 8 – 6 ppm; carbohydrates/alcohols: 6 – 4 ppm; O-alkyls: 4 – 3 ppm and alkyls: 3 – 0 ppm (Dellagreca et al. 2002, 2011).

Soil biological analyses

The biological analyses, which were performed in triplicate within a week after sampling on fresh samples stored at 4°C, included microbial and fungal biomasses and soil basal respiration (R). The microbial biomass was evaluated as microbial carbon (C_{mic}), according to Anderson and Domsch (1978), by the method of substrate-induced respiration (SIR). SIR was determined using glucose 1% as the substrate and the evolved CO_2 in a 72-h incubation at 25°C in the dark (Anderson and Domsch 1978). The evolved CO_2 was adsorbed in NaOH and measured by two-phase titration with HCI (Froment 1972). The fungal biomass was assayed by the membrane filter technique (Sundman and Sivelä 1978). After staining with aniline blue, hyphal length was determined by the intersection method (Olson 1950) with an optical microscope (Optika, B-252). The results were expressed as fungal carbon (C_{fung}) on the basis of the mean values reported for the C/N ratio (Killham 1994) and N content (Swift et al. 1979) in fungi. R, expressed as mg $CO_2 g^{-1}$ d.w., was determined by measuring the CO_2 evolved in a 10-day incubation at 25°C in the dark (Alef 1995).

Microbial and fungal carbon and basal respiration were expressed as fractions of organic carbon and were indicators of soil organic matter turnover and stability (Barcena et al. 2014).

Statistical analyses

The means and standard errors of measured variables were calculated for stands (plant cover and elevation) and for soil layers (organic layers and mineral soil). Data were checked for normality and heteroscedasticity, and when necessary, they were transformed. Two-way ANOVA followed by Holm-Sidak post hoc tests (p < 0.05) was used to assess the significance of the differences among stands according to plant cover (P and S) and elevation (L and H).

The statistical analyses were performed using Systat_SigmaPlot_12.5 software (Jandel Scientific, San Jose, CA, USA).

Results

Pine forest and shrubland soil properties

Pine forests and shrublands showed differences in the main characteristics of mineral soil and organic horizon (Tables 1, 2 and Fig. 1). In detail, plant cover significantly affected only the water holding capacity (WHC) and organic matter content (OM), which showed the highest values in the pine forest soils (Table 1). Indeed, significantly different trends were shown by the mass of the organic horizon, pH,

bulk density, WHC, OM and C/N ratio in soils under pine and shrubs according to elevation (Table 1, Supplementary material S1, S2).

Table 1

Stand description and main characteristics of mineral soil (0–10 cm) of the investigated sites with different plant cover (Pine forest-P; Shrubland-S) inside the Vesuvius National Park at low (L) and high (H) altitudes. Data are means (P and S: n = 16; L and H: n = 8) \pm standard error. Different lowercase letters indicate a significant difference among sites according to plant cover and altitude; different uppercase letters indicate a significant difference between average values recorded in Pine forest and Shrubland (two-way ANOVA, P < 0.05).

| | Lat/Long | Altitude | Organic horizon mass | рН- Н ₂ 0 | Bulk density | WHC* | OM** | C/N |
|--|----------------------------------|----------|----------------------------|-------------------------|-------------------|--------------------|-------------------|--------------------|
| | | m a.s.l. | g m ⁻² | | g m ⁻³ | % d.w. | % d.w. | |
| Pine forest-P | | | 1870.9± 378.9A | 6.9 ± 0.3A | 0.3 ± 0.03A | 78.5 ± 12.6A | 10.6 ± 2.1A | 28.2 ± 10.4A |
| Low altitude (LP) | 40°48'19.04"N 14°26'13.361"E | 600 | 999.0 ± 153.7c | 6.5 ± 0.3b | 0.2± 0.01b | 115.2 ± 6.9a | 15.9 ± 1.3a | 46.5 ± 5.3a |
| High altitude (HP) | 40°48'55.246"N 14°26'18.679"E | 900 | 2742.8 ± 278.2a | 7.4 ± 0.3a | 0.3 ± 0.04a | 41.9 ± 1.8c | 5.4 ± 1.4c | 10.0 ± 4.1b |
| Shrubland- S | | | 1752.2± 197.4A | 7.0 ± 0.0.2A | 0.3± 0.1A | 55.2 ± 13.4B | 6.7 ± 1.2B | 27.2 ± 9.1A |
| Low altitude (LS) | 40°49'49.156"N 14°24'0.273"E | 600 | 1803.3 ± 340.2b | 7.1 ± 0.4a | 0.2 ± 0.03b | 36.1 ± 1.1d | 4.7 ± 0.4c | 37.2 ± 6.5a |
| High altitude (HS) | 40°49'51.935"N 14°25'28.606"E | 900 | 1700.8 ± 339.8b | 6.9 ± 0.1ab | 0.3 ± 0.03ab | 74.3 ± 8.6b | 8.7 ± 1.0b | 17.3 ± 4.5b |
| * WHC: Water Holding Capacity;** OM: Organic Matter; | | | | | | | | |

Table 2

Total Carbon content (mg g-1) and fractions (% total C) of the major types of carbon functional groups (Alkyl C, Methoxyl C, O-Alkyl C, Aryl C, Phenol C, and Carboxyl C) in the organic horizon (OL and OF + OH layers) from Pine Forest (P) and Shrubland (S) at Mount Vesuvius. Carbon data were analyzed through solid state 13C Nuclear Magnetic Resonance (NMR) and chemical shift ranges in ppm are reported in parenthesis. Different lowercase letters indicate a significant difference among sites according to plant cover and elevation (two-way ANOVA, P < 0.05). Different uppercase letters indicate a significant difference between average values recorded in Pine forests and Shrublands (two-way ANOVA, P < 0.05).

| Stand | Carbon mg g ⁻¹ | Carboxyl C (200 – 160 ppm) | Phenol C (160 - 145 ppm) | Aryl C (145 – 110 ppm) | 0 – Alkyl C (110 – 60 ppm) | Methoxyl C (60 – 45 ppm) | Alkyl C (45 – 0 ppm) |
|---------------------------|------------------------------|--|--------------------------------------|---------------------------------|----------------------------------|--------------------------------|----------------------------------|
| Pine forest-OL | 521.3 ± 4.1A | 4.6 | 7.3 | 10.8 | 54.7 | 8.1 | 14.5 |
| Low elevation (LP) | 518.2 ± 3.9a | 5.0 | 8.1 | 10.2 | 53.6 | 8.4 | 14.7 |
| High elevation (HP) | 524.4 ± 8.7a | 4.1 | 6.6 | 11.4 | 55.8 | 7.9 | 14.3 |
| Shrubland- OL | 384.63 ± 33.9B | 6.6 | 7.8 | 9.9 | 49.9 | 6.7 | 19.2 |
| Low elevation (LS) | 342.6 ± 32.3b | 5.9 | 8.6 | 10.2 | 50.1 | 6.5 | 18.8 |
| High elevation (HS) | 426.7 ± 13.9b | 7.1 | 7.1 | 9.8 | 49.8 | 6.8 | 19.5 |
| Pine forest -OF + OH | 302.6 ± 17.9A | 5.8 | 7.9 | 9.5 | 53.6 | 8.7 | 14.6 |
| Low elevation (LP) | 344.2 ± 16.5a | 5.4 | 7.7 | 10.1 | 52.1 | 7.0 | 17.7 |
| High elevation (HP) | 261.1 ± 18.0b | 6.4 | 8.2 | 8.7 | 55.7 | 11.0 | 10.4 |
| Shrubland -OF + OH | 175.7 ± 12.9B | 8.4 | 9.3 | 9.3 | 53.1 | 9.3 | 10.6 |
| Low elevation (LS) | 171.2 ± 17.3c | 7.9 | 8.6 | 8.5 | 56.7 | 9.2 | 9.2 |

| Stand | Carbon mg g ⁻¹ | Carboxyl C (200 - 160 ppm) | Phenol C (160 – 145 ppm) | Aryl C (145 - 110 ppm) | 0 – Alkyl C (110 – 60 ppm) | Methoxyl C (60 – 45 ppm) | Alkyl C (45 – O ppm) |
|---------------------------|------------------------------|--|--------------------------------------|---------------------------------|----------------------------------|--------------------------------|----------------------------------|
| High elevation (HS) | 180.3 ±15.6c | 8.9 | 10.0 | 10.1 | 49.8 | 9.4 | 11.9 |

The soils of pine forests at low elevation showed the lowest values of accumulated organic mass, pH and bulk density and the highest values of WHC, OM and the C/N ratio (Table 1, Supplementary material S1, S2).

The soils of shrublands at low elevation had lower WHC and OM and higher pH and C/N ratio than soils at high elevation (Table 1, Supplementary material S1, S2).

Carbon and nitrogen pools in pine forests

Along the pine forest profile, the upper organic layers (OLs) and the surface mineral soils (0–10 cm) had C and N pools (g m²) that were larger than those of shrublands (Fig. 1, Supplementary material S1 and S2).

In pine forests, only the mineral soils showed significant differences in the C pool according to elevation, with higher values at low elevation than at high elevation (Fig. 1, Supplementary material S2).

Moreover, the fermentation and humic layers (OF + OH) of pine forests had significantly poorer N pools than those of shrublands (Fig. 1). Additionally, the pine organic layer (OL) showed the largest N pool at low elevation, while the OF + OH layer and mineral soil had the largest N pool at high elevation.

In pine forests, the chemical composition of the organic horizon (OL and OF + OH), analysed as the total C content and as the mass of the major types of carbon functional groups (C-bonds, De Marco et al. 2012) through solid-state ¹³C NMR, showed higher values for C content and for phenol C, aryl C, O alkyl C and methoxyl C than those of shrublands (Table 2, Supplementary material S1, S3). If C-bonds are reported as a fraction of the total C content, the pine organic layer is poorer in carboxyl C and phenol C than shrublands (Table 2).

In the OL layer of pine forests, carboxyl C and phenol C had higher values at low elevation than at high elevation; in contrast, aryl C and O-alkyl C had higher values at high elevation (Table 2, Supplementary material S3). In the OF + OH layer, pine forests showed higher C, aryl C and alkyl C contents at low elevation (Table 2, Supplementary material S3).

In mineral soils, the C and N pools were analysed as the total C and N contents and also as soluble fractions (Fig. 2). The soil C and N contents in the pine forests were significantly higher than those measured in the shrublands soil, while the soluble component did not seem to be directly affected by the

plant cover (Fig. 2, Supplementary material S3). Regarding elevation, the pine forests soil at low elevation had the highest C content and the lowest N content and soluble C fraction (Fig. 2, Supplementary material S3). The chemical composition of soil water extracts, analysed (Table 3, Supplementary material S2) through the ¹H NMR spectra, highlighted a significantly higher content of carboxylic acids and aromatic compounds and a lower content of alkyls in pine forests than in shrublands. In the soil of pine forests, water extracts were richer in carboxylic acids and O-alkyls and poorer in aromatic compounds at low elevation than at high elevation (Table 3, Supplementary material S2).

Table 3

Components of water extracts from the mineral soil of Pine forest (P) and Shrubland (S) at Mount Vesuvius.. Data are presented as percent of total area for ¹H-NMR spectra (chemical shift ranges in ppm are reported in parenthesis). Different lowercase letters indicate a significant difference among sites according to plant cover and elevation (two-way ANOVA, P < 0.05). Different uppercase letters indicate a significant difference between average values recorded in Pine forests and Shrublands (two-way ANOVA, P < 0.05).

| Stand | Carboxylic ac/ Aldehydes (12 – 8 ppm) | Aromatic compounds (8 – 6 ppm) | Carbohydrates/ Alcohols (6 – 4 ppm) | 0 – Alkyls (4 – 3 ppm) | Alkyls (3 – 0 ppm) |
|---------------------------|--|--------------------------------------|---|---------------------------------|--------------------------|
| Pine forest- P | 9.1 ± 1.5A | 11.5±0.5A | 51.4±1.1A | 8.1 ± 0.4A | 19.9 ± 0.6B |
| Low elevation (LP) | 11.3±1.4a | 10.8 ± 0.5b | 50.0 ± 1.6a | 8.8 ± 0.1b | 19.1 ± 0.5c |
| High elevation (HP) | 6.8 ± 1.5b | 12.3 ± 0.3a | 52.7 ± 0.8a | 7.5 ± 0.4c | 20.7 ± 0.7b |
| Shrubland- S | 5.4±1.2B | 7.5±1.1B | 47.4±1.6A | 9.0 ± 1.3A | 30.7 ± 2.6A |
| Low elevation (LS) | 3.7±0.4c | 5.7 ± 0.5c | 43.6 ± 2.2b | 6.7 ± 0.2c | 40.3 ± 1.1a |
| High elevation (HS) | 7.1 ± 1.8b | 9.2 ± 1.0b | 51.2 ± 2.3a | 11.3 ± 1.0a | 21.1 ± 0.5b |

In addition, the pine forest and shrubland soils showed similar average values for microbial and fungal C and microbial respiration (Fig. 3, Supplementary material S2). Regarding elevation, the pine forest soils showed higher C_{mic} at high elevation than at low elevation (Fig. 3, Supplementary material S2).

Carbon and nitrogen pools in shrublands

As shown in the previous section, the shrublands profiles were poorer in C and N in the OL layer and mineral soil and richer in N in the OF + OH layer than those in the pine forests. However, different trends were recorded regarding elevation.

Specifically, the shrublands organic horizon (OL and OF + OH) showed the highest values of C and N pools at high elevation (Fig. 1, Supplementary material S1, S2). In mineral soil, only the N pool had significantly higher values at high elevations than at low elevations (Fig. 1, Supplementary material S1, S2).

No difference was observed for the total C content in the organic horizon of the shrublands based on elevation (Table 2, Supplementary material S1). The organic horizon of shrublands at high elevation was richer in carboxyl C and alkyl C fractions than that at low elevation (Table 2, Supplementary material S1, S3). Furthermore, in the OL layer, the fractions of phenols and aryls of the total C were lower at high elevations than at low elevations, while the opposite result was found for the OF + OH layer (Table 2).

The shrublands soils at low elevation showed the lowest C and N values (Fig. 2) and the highest soluble N fractions (Fig. 2, Supplementary material S2). The ¹H NMR spectra highlighted that in low-elevation shrublands, soil water extracts had a high amount of only alkyls and were poorer in all the other components, opposite to the high-elevation shrublands (Table 3, Supplementary material S1).

In contrast to pine forests, microbial and fungal carbon and basal respiration showed great variability with elevation in shrublands. In shrublands soils, C_{mic} and C_{fung} were higher at low elevation, and R was higher at high elevation (Fig. 3, Supplementary material S2).

Discussion

Our results confirm that afforestation with pine, carried out to consolidate mountain slopes and reduce erosion phenomena, induced profound changes in the soil characteristics and C sequestration rate, which was in accordance with previous research results (Ono et al. 2011; Deng et al. 2017). In fact, in the investigated Mediterranean area, pine forests significantly increased the amount of soil OM and the soil C and N pools compared to shrublands. Thereby, pine species (*Pinus* spp.) play a dominant role in the organic matter sequestration rate of top mineral soil not only in boreal and temperate forests (Berg and McClaugherty 2020) but also in Mediterranean ecosystems. In the studied areas, pine forests and shrublands showed similar values of mass accumulated in the organic horizon and C/N ratio (indicator of litter quality), but we can assume there were differences in the mineralization and humification processes and in the stability of the accumulated organic residue. The steadiness of aromatic and alkyl fractions in the organic horizon of pine forests and the low N pools in the OF + OH layer could suggest a higher humification degree and greater stability of soil organic matter in these forests than in shrublands (Berg 2018; Ono et al. 2011). However, in forest soils, the alkyl component of organic matter tends to be more protected from leaching and microbial attack; therefore, it is less soluble in water (Schöning et al. 2005). In addition, the abundance of aromatic and carboxylic components of the soil soluble fraction of

pine forests could be associated with the higher presence of humic and fulvic acids in these soils than those in shrublands (Dai et al. 2001).

The role of litter amount and quality and of its variations in the organic horizon were confirmed by the different trends highlighted for the C and N pools in the pine forests and shrublands based on elevation. In fact, climatic conditions, mainly temperature and humidity, can be regulating factors for both litter production and the amount of labile organic fraction and recalcitrant residues, affecting the C sequestration rate and soil organic matter stability (Berg and Meentemeyer 2001; Liu et al. 2003).

According to Memoli et al. (2019) and Panico et al. (2020), the higher temperature at low elevation than at high elevation that characterize Vesuvius slopes could have strongly influenced the soil characteristics and process rates and be at least partly responsible for the highest soil OM contents and C pools in lowelevation pine forests and in high-elevation shrublands. The important role of temperature appeared to be confirmed by the clearly significant and positive relationship between the C sequestration rate and mean annual temperature found for different European (North and South Europe) coniferous stands by Berg and McClaugherty (2020).

In low-elevation pine forests, a significantly lower mass was accumulated in the organic horizon than at high elevations, but no difference in C pools was found along the organic horizon (from the OL to the OF + OH layers) with elevation. In contrast, variations in the chemical composition of organic detritus were visible, and changes in the C fractions along the pine organic horizon at low and high elevations could mean different decomposition patterns and humification stages, as described by De Marco et al. (2012). Specifically, the decrease in aromatic compounds (phenol C + aryl C + methoxyl C) and O-alkyl C (carbohydrates as cellulose) from OL to OF + OH and the respective increase in aliphatic compounds (alkyl C) at low elevations may be associated with humification processes according to the studies by Ono et al. (2009) and Osono et al. (2008). Furthermore, the high stability of soil organic matter in lowelevation pine forests was confirmed by the higher C/N ratio, which was almost 5 times higher than that in high-elevation pine forests. In addition, the soluble component of C was significantly lower at low elevation and was rich in carboxylic acids and O-alkyl groups, *i.e.*, oxidized compounds and products of previous litter degradation processes (De Marco et al. 2012). Conversely, at high elevations, the observed aromatic compound accumulation (from 26-28% in the OL layer and OF + OH layer, respectively) could be related to the initial and middle stages of the decomposition process, according to data reported by Berg and McClaugherty for conifer forests (2020).

Similar to pine forests, shrublands seemed to show a slower decomposition of organic detritus at high elevations than at low elevations, likely due to a greater presence of recalcitrant compounds, which tended to accumulate in the fermentative layer. In fact, in high-elevation shrublands, the increase in the C pool along the organic horizon (from OL to OF + OH) was particularly high compared to what happened in pine forests (6 times in pine and 13 times in shrubs) and the OF + OH layer was particularly rich in aromatic compounds that represented 30% of C compared to only 24% in OL layer. Our results seem to be in agreement with Osono et al. (2008), who showed that Japanese forests of *Swida controversa* and

Fagus crenata had different decomposition patterns related to elevation for the two species and observed a different accumulation of aromatic compounds due to a selective preservation of lignin during decay.

In addition, in pine forests and shrublands, the build-up of the humus layer and the stability of SOM are differently affected by the N concentration of the organic debris. In both pine forests and shrublands, the N concentration in OL layers affects its fate, favouring its release or immobilization in the respective OF + OH layers and mineral soils. Therefore, the high N content in OL layers in low-elevation pine forests could account for its mineralization and reduced content in the underlying soil layers according to Hasegawa (2004) and Mooshammer et al. (2012) for different litter types. Moreover, Berg (2018) reported that litter N mineralization could be related to faster decomposition and humification processes as well as more stable organic material accumulation in the soil.

In contrast, for high-elevation pine forests and shrublands, low N concentrations in the OL layer induced immobilization of this element in the fermentation layers, which may impede the degradation of lignified tissue in the late decomposition stage (Berg and Ekbohm 1991; Knicker 2004). Several studies confirmed that a high N concentration in litter favours the formation of stable complexes that slow decomposition at late stages and improve C accumulation on the forest floor (De Marco et al. 2016; De Marco et al. 2021; Fioretto et al. 2005; Michel and Matzner 2002).

However, the N fraction, as an important biogenic element in the process of soil microbial growth, can enhance the ability of microbes to utilize C sources and promote an increase in microbial biomass (Demoling et al. 2008). According to Xiang et al. (2017), in pine forests, the microbial biomass and, in detail, the bacterial component are influenced by the availability and the input of resources in the organic layer that are particularly abundant at high elevation (high mass in the organic horizon). In addition, in low-elevation pine forests and in high-elevation shrublands, a decrease in the bacterial component could be due to the lower capability of bacteria to exploit more recalcitrant compounds than the fungal component (De Marco et al. 2018; Memoli et al. 2018). Therefore, the assimilation efficiency of C and N is different among different soil microorganisms, and the soil microbial community is modulated by the availability and transformation of C and N (Shao et al. 2019; Zhang et al. 2018). The microbial community structure can characterize the soil C sequestration level and altered C cycling patterns (Malik et al. 2016). Generally, an increase in fungi in microbial biomass indicates improvements in soil C sequestration capacity and high stability in terms of C pools (Bardgett et al. 2001; Boyle et al. 2008). In the present study, the C_{fung}/C_{mic} values were highest in low-elevation pine forests (0.25 and 0.08 at low and high elevations, respectively), confirming a positive effect of pine afforestation at low elevations on C conservation and stability (Liu et al. 2018; 2019).

In addition, the different microclimatic conditions related to different elevations can, together with the lack of nutrients such as N, affect the presence and activity of fungi and bacteria. At high elevations, the microbial and fungal biomasses of the shrublands soil were significantly reduced compared to those at low elevations, despite being more active. The higher microbial respiration in shrublands soil at high elevations than at low elevations can be a response of the microbial community to stress conditions that

stimulate the use of C resources (Liu et al. 2019; Panico et al. 2020). Moreover, in low-elevation shrublands, the chemical composition of the soluble fraction seemed to confirm the higher resistance to oxidation given the high values of saturated compounds such as alkyls. This complexity and recalcitrance may also explain the lowest values of microbial respiration (Yüksek et al. 2011).

Conclusion

In conclusion, coniferous tree species sequester C faster than shrubs and herbaceous species especially at low elevation under favourable microclimatic conditions with higher temperatures. In pine forests, microclimatic conditions related to elevation drive the C sequestration rate and humification degree, suggesting that afforestation with pine in Mediterranean areas at low elevations could favour the long-term soil accumulation of stable organic matter. In addition, in both pine forests and shrublands, N appears to strongly affect litter mineralization, humification and the C sequestration rate. In pine forests, the higher concentration of N in the upper organic layer speeds up N loss in the fermentative layer and stimulates humus formation and C accumulation at low elevations; in shrublands, the initial low concentration at low elevations leads to N immobilization in the fermentative layer and a slowdown in soil C accumulation.

The soil C and N pools reflect changes in the chemical composition of the upper organic layers and of soil soluble organic matter. Such changes highlighted by nuclear magnetic resonance spectroscopy can clarify patterns of decay processes and help to make predictions in a climate change scenario.

Therefore, elucidating SOC and N dynamics in soils under different plant cover and microclimatic conditions has important implications for the sustainable management of land resources and predictions of future global C and N cycles.

Declarations

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Availability of data and material Data available on request

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Authors' contributions GM, ADM and RB conceived and designed the experiments; AZ performed the NMR measurements and analyzed the data; GM, ADM, SCP, VM, LS performed field work and contributed to

analyses; ADM, LS and VM wrote the paper. All authors contributed to the article and approved the submitted version.

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Figures



Figure 1

C and N pools (g m-2) in the organic horizon (OL and OF+OH) and mineral soil (0-10 cm) of Pine Forests (P) and Shrublands (S) at low (L) and high (H) elevation along Mount Vesuvius slopes. Values are means ± standard error. Different lowercase letters indicate a significant difference among sites according to plant cover and elevation (two-way ANOVA, P<0.05). Different uppercase letters indicate a significant difference axis and average values recorded in Pine forests and Shrublands (two-way ANOVA, P<0.05)



Figure 2

Total (mg g-1 d.w.) and soluble (% total) C and N content in the mineral soil of Pine Forests (P) and Shrublands (S) at Mount Vesuvius. Values are means ± standard error. Different lowercase letters indicate a significant difference among sites according to plant cover and elevation (two-way ANOVA, P<0.05). Different uppercase letters indicate a significant difference between average values recorded in Pine forests and Shrublands (two-way ANOVA, P<0.05)



Figure 3

Microbial (Cmic) and fungal carbon (Cfung) and basal respiration (R) per gram of organic C (mg g OC-1) and in the mineral soil of Pine Forests (P) and Shrublands (S) at Mount Vesuvius. Values are means ± standard error. Different lowercase letters indicate a significant difference among sites according to plant cover and elevation (two-way ANOVA, P<0.05). Different uppercase letters indicate a significant difference between average values recorded in Pine forests and Shrublands (two-way ANOVA, P<0.05)

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