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RELATIONSHIPS BETWEEN HUMUS PROFILES AND C CYCLING, FIRST RESULTS FROM A MEDITERRANEAN PINE FOREST

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Abstract

Forest degradation may reduce the forest potential to act as a C sink, or even increase C losses through greenhouse gas (GHG) emissions. Microbial processes strongly affect soil organic matter (SOM) decomposition and therefore C fluxes. Since the first and most important modifications of microbial processes occur within humus profiles, humus characteristics and structure might affect microbial processes and GHG emissions. The study was carried out to relate C cycling in a degraded pine forest with the morphology and characteristics of the humus profiles. The study area was located near Florence (Central Italy) in a reforest area planted with Black pine, Brutia pine, and Cypress. In spring 2016, 9 humus profiles were described, sampled and classified, and CO_2 and CH_4 fluxes were measured. Humus profiles were analyzed for bulk density, particle size, N, C, lime content and pH. Bio macroaggregates of the first mineral horizon were separated by moist sieving in three size classes (<1 mm, 1-4 mm, >4 mm) for the humus classification. Statistical relationships were checked by a Spearman test. Eumacroamphi was the main humus form, while Dysmull and Pachiamphi were less frequent. There were significant correlations between CH_4 fluxes and both the thickness of the transition from organic to inorganic horizons, and the percentage of aggregates in different size classes. CO_2 emission did not provide significant correlations with humus features. These first results suggest that the activity of methanotrophic bacteria responsible for CH_4 uptake might be lower where the thickness of the transition between the organic and mineral horizons is larger. Thus, the accumulation of SOM in between the organic and mineral horizons would lower the functionality of methanotrophic bacteria and reduce the soil GHG sequestration potential.

Keywords: *humus profile, soil, GHG, organic carbon, methane*

Introduction

In the Mediterranean basin since the late 19th century coniferous species have been often used for land restoration. Nowadays, most of these pine stands are concluding their role of pioneer species and pine plantations show degradation symptoms with

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many dead, fallen and/or damaged trees. In Italy in particular, 462,568 ha, that is the 31% of pine forests in the Mediterranean zone, show degradation symptoms or are damaged (Gasparini and Tabacchi 2011**)**.

Forests are considered an important sink for greenhouse gases (GHGs), and previous studies investigated the carbon (C) sequestration in soils affected by coniferous reforestation (eg. Di Biase et al., 2005). However, forest health may strongly affect this function. Forest degradation leads to a decrease in canopy cover and regeneration, as well as to coverage fragmentation, which affect the annual increment of C sequestration. Then forest degradation contributes to atmospheric GHG emissions through decomposition of fallen plant material and soil C release. C emissions from deforestation and forest degradation have been estimated to account for about 12-20% of global anthropogenic CO_2 emissions (IPCC, 2007).

Better understanding C cycling and processes involved is fundamental to link pools, fluxes and transformations occurring at the interface between vegetation and soil. The first and most important soil organic matter (SOM) decomposition processes occur within humus profiles, carried out by biological (micro-, meso- and macro-fauna, as well as microflora) activity (Zanella et al., 2017). The microbial and faunal actors of decomposition processes are tightly interconnected (Ponge, 2013). The organic matter of humus and of the transition layers is incorporated into the excrements from epigeic earthworms, enchytraeids and microarthropods. Their droppings constitute a stable stock, not readily accessible to microorganisms. They correspond to the stable humus of the slow-decomposing organic matter remaining after decomposition, described by several authors (a.o., Kögel-Knabner and Matzner, 2008, Kleber et al., 2011). The same processes regulate the direction and intensity of GHG fluxes (CO_2 and CH_4), being the final outcome of a complex interplay among several components of biological activity (Trueman and Gonzalez-Meler, 2005). Indeed, soil CO_2 efflux is the result of autotrophic (root and rhizosphere) and heterotrophic respiration deriving from the activity of a multi-organism network decomposing soil-derived C (Kuzyakov, 2006). These relationships represent also the driving forces of humus profiles differentiation, characteristics and structure. Therefore, we hypothesized that diverse humus forms and properties reflect differences in CO_2 and CH_4 fluxes. Some authors evaluated the different humus forms as an indicator of ecosystem conservation level, especially for stressed vegetation (Mallik and Newton, 1988, Topoliantz and Ponge 2000, Klinka et al., 1900). Recently, humus forms have been also taken into account as indicators of soil organic C storage (Andreetta et al. 2011, De Vos et al., 2015). Humus forms are intimately linked to soil fauna and bacteria activity (Wallwork, 1970; Ponge and Delhaye, 1995**;** Ponge et al., 1997**,** Coleman et al., 2004; Salmon et al., 2006**;** Galvan et al.2008; Carletti 2009; Zanella et al., 2011a), however, the importance of the forest floor in C and nutrient cycling and consequently on CO_2 storage is commonly undervalued (Aaltonen et al. 2011). Ponge (2013) indicated the humus forms, including litter, as the place where interactions between aboveground and belowground biodiversity occur. Halmeenmäki et al., (2015) recently investigated the methanogen activity in the above- and below-ground compartments in a boreal Scot pine forest. Sanhueza et al. (1998) and Dong et al. (2002) investigated the effects of the leaves and the humus material on GHG fluxes from the soil in a temperate, deciduous forest.

However, no studies addressed the linkage between diverse humus functionalities and GHG fluxes in degraded forests. This paper represents an innovation with respect to previous studies, adding a first insight on the relationships between the morphology and characteristics of the humus profile and C cycling, in particular CO_2 and CH_4 fluxes, in a degraded pine forest. These results are important to understand the interplay between GHG emissions, SOM decomposition, and aggregate formation in forest soils. They contribute to the further use of humus profile description as an indicator of environmental processes, and can provide suggestions for the selection of the most appropriate silvicultural practices to reduce GHG emission and increase C sequestration potential.

Materials and Methods

Studied area

The study area is located in Italy, in the peri-urban forest of Monte Morello (43°51’20’’N; 11°14’23’’E), immediately North-West of the urban area of Florence. The forest has derived from reforestations realized over a surface of around 1,035 ha from 1909 to 1980. The previous land use was degraded pastures and shrub lands. The main tree species used in the reforestation project were black pine (*Pinus nigra* J.F.Arnold), Brutia pine (*Pinus brutia* Ten. subsp. brutia), and cypress (*Cupressus* spp.). At the moment, Monte Morello forest is a mixed forest dominated by coniferous and with the presence of deciduous species, mainly Turkey oak (*Quercus cerris* L.), Downey oak (*Quercus pubescens* L.) and Flowering ash (*Fraxinus ornus* L.). The vegetation can be considered a degraded forest often characterized by poor regeneration, marked susceptibility to adversities, huge quantity of deadwood and a high degree of flammability (Cenni et al., 1998; Nocentini, 1995).

The altitude of the studied plots ranges from 590 to 650 a.s.l.. The precipitations are concentrated from autumn to early spring. July is the driest month, while October and November are the rainiest. During the last decades (1980s -2010) the total annual rainfall was 1,003 mm and the average annual temperature 13.9°C. The soil thermometric regime is mesic and the soil moisture regime is udic, while the soil aridity index (SAI) ranges between 36 and 51 (mean annual number of days with dry soil, Costantini et al., 2009). From a geomorphological point of view, the substratum is calcareous flysch (turbidites), constituted by alternating limestones, marly limestones (“alberese”) marls, claystones and, subordinately, sandstones.

GHG emissions measurements

Gas sampling was conducted in nine plots of about 1 ha, with 2 replicates for each plot in 9 sampling dates during 2016. Sampling performed in April was concurrent with humus profile description. 18 vented closed opaque chambers (30 cm in diameter) made of polyvinyl chloride, sat on a base collar that had been inserted into the soil. Chamber lid was covered with a reflective insulation and equipped with vent tubes, fans to mix headspace air, gas sampling ports, and thermocouple wire to measure chamber air temperature. During each gas sampling event, chambers were closed for 30 min and gas samples were collected at 0, 10, 20, and 30 min. Headspace gas samples were obtained using an air-tight 30-mL propylene syringes and immediately pressurized into pre-evacuated 12-mL glass Exetainer® vials (Labco Ltd., UK).

The gas samples were analyzed with a GC-2014 gas chromatograph (GC) (Shimadzu Scientific, USA) equipped with a thermal conductivity detector (TCD) for CO_2, a ^{63}Ni electron capture detector (ECD) for N_2O and a flame ionization detector (FID) for CH_4. The GC detection limits were 8357 ng L^{-1} for CO_2, 11.6 ng L^{-1} for N_2O and 84.8 ng L^{-1} for CH_4. Chamber gas concentrations were converted to mass per volume unit using the Ideal Gas Law and measured chamber air temperatures and volumes. Fluxes were calculated using the slope of linear regression of gas concentration vs. chamber closure time and the enclosed soil surface area. Fluxes were set to zero if the change in gas concentration during chamber enclosure fell below the minimum detection limit of GC, and flux values were rejected (i.e., treated as missing data) if they passed the detection test but had a coefficient of determination (R2) < 0.75.

Soil sampling, analysis, and classification

A humus profile corresponds to the portion of topsoil strongly influenced by organic matter and it is composed by different combinations of the organic layers OL, OF and OH, the organo-mineral horizon A, and the mineral E horizon, when present (Zanella et al. 2011a). In the study area, after a preliminary soil survey, nine humus profiles, placed close to the GHG monitoring sites, were dug, described and sampled in the early spring of 2016, at soil water conditions close to field capacity. Classification followed the European system proposed by Zanella et al. (2011b). This classification agrees with the recent international soil classifications, since the old and new European and North American systems of humus forms classification are taken into account and adapted to the European ecological conditions. The classification is based on morphogenetic descriptions and diagnostic horizons. Specifically, a limit of 20% w/w organic carbon (OC) distinguishes O horizons from organo-mineral horizon (IUSS, 2007).

Among the O horizons, the OL, OF, OH are characterized by different level of alteration of the vegetation debris. OL is mainly made up by leaves and needles, twigs and woody materials easily discernible to the naked eye. In OLn, the new litter (age < 1 year), is the no transformed or discolored materials, while in OLv the old litter (aged more than 3 months) and slightly altered fragmented leaves and/or needles constitute the layer. OF shows the accumulation of partly decomposed litter, debris of leaves/needles, twigs and woody materials, but without any entire plant organ. OH is the humified material, where the transformed OM accumulates because of the action of soil macro- and micro- organisms.

The first organo-mineral A horizon was sampled and described for the main physical aspects: superficial stoniness and rock abundance, coarse material, humid colour, relationship between organic and mineral materials, aggregates, pores, roots, fungi, and macro evidences of biological activity, e.g., earthworms' channels and droplets, arthropods. A specific care was given to the measurement of the size of the boundary between organic and organo-mineral horizons, as requested by the humus classification.

Dominant ped size was first identified in the field observing the soil mass by the naked eye, and then measured in laboratory. Moist soil aggregates of less than 2 cm were separated with two sieves at 4 and 1 mm, which correspond to the limits between biomacro, biomeso and biomicro aggregates. The samples were gently manipulated to separate the original peds. After drying, the coarse material (>2 mm) was removed by the fractions, the dry aggregated fractions were weighted, and the percentage of biomacro, biomeso and biomicro soil aggregates of the organo-mineral horizon A was calculated.

The horizons were classified on the bases of the dominant biomacrostructure. Biostructures have different origin. Biomacrostructured A horizons are dominated by aneci-endovermic activity; biomesostructured A by endo-epivermic activity; and biomicrostructured A by enchy-arthropodic activity (Zanella et al. 2011a).

Total organic content C (TOC) and total N (TN) contents in the bulk soil and dry A aggregates were measured by dry combustion on a Thermo Flash 2000 CN soil analyzer (Thermo Fisher Sci.). To this aim, 20 to 40 mg soil were weighed into Ag-foil capsules and a pre-treatment with 10% HCl until complete removal of carbonates was applied for TOC determination. Non pre-treated samples were used for total C (TC) determination. Carbonates have been estimated by the difference between TC and TOC in each sample. The first 30 cm of mineral horizons (under the O horizons) were sampled and analyzed at each plot, at 0-10 and 10-30 cm depth. Soil pH was determined in water (1:2.5 soil: water ratio). Particle size analysis was carried out following the hydrometer method (Gee and Bauder, 1986). Soil bulk density (BD) was determined from undisturbed 100 cm^3 soil cores collected with a hammer-driven liner sampler (Eijkelkamp, The Netherlands). The samples were dried at 105 °C until constant weight and the BD calculated by the ratio between the dry weight and the soil core volume (Blake 197et al., 1986). Cation exchange capacity (CEC) was determined following Gillman (1979): 2 g of soil were saturated with a 10% $BaCl_2$ solution pH 8.1. After 2 hours shaking, samples were centrifuged and solution was decanted. 0.1N $MgSO_4$ was then added to replace Ba with Mg. After shaking, samples were centrifuged again and solution was decanted. 10ml of supernatant were mixed with NH_4Cl pH 10 and then titrated with EDTA 0.05N with a potentiometric titration. Carbon stock was calculated for each horizon on the basis of TOC content, bulk density, coarse fragments for the first 30 cm of the mineral soil. Bulk density was measured for the first 10 cm and estimated for the deeper horizon on the basis of a linear regression with sand and TOC content.

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Three out the nine plots were selected as representative of the studied stations and soil profiles were dug and described to about 150 cm. Filed soil description followed the national soil survey manual (Costantini, 2007). The sampled horizons were analyzed as for pH, TOC, TN, and carbonates. Soils were finally classified according to the World Reference Base for soil Resources (IUSS, 2014).

Statistical analyses

A descriptive statistic was carried out for the pedological characterization. Correlation matrix was calculated using data on C fluxes, humus genetic features and main pedological features of the plots The Spearman rank R test allowed a nonparametric correlation. The p-level for highlighting correlation was established to 0.1, in order to get possible trends that may be meaningful though not statistically strong, due to the limited number of cases.

Results

Main soil features

The studied soils showed moderately alkaline reaction, usually high and very high carbonate content and very high organic carbon content in the first 30 cm. Bulk density in the first 10 cm ranged from 0.85 to 1.05 g cm^{-3} except for plot 9 (0.6 g cm^{-3}), and texture was always loam or clay loam.

Table1. *Pedological features of the plot.*

Plot/ Humus	D/C (%)	S/R/G (%)	depth (cm)	TN (g kg^{-1})	TOC (g kg^{-1})	$CaCO_3$ (% w/w)	C/N	BD (g cm^{-3})	CEC (cmol kg^{-1})	pH	Sand (g dag^{-1})	Clay (g dag^{-1})	AWC (mm/m)
1/E	1/99	10/0/10	10	4.68	54.1	6.72	11.6	0.98	38.2	8.1	33	43	62
			30	2.80	24.5	11.76	8.75		36.5	8.2	37	25	
2/E	5/95	5/0/25	10	3.62	54.5	1.51	15.1	1.05	66.5	8.3	34	29	64
			30	2.42	21.0	11.94	8.68		35.7	8.2	34	25	
3/E	90/10	25/0/30	10	4.61	49.6	0.66	10.8	0.89	44.5	7.9	39	27	65
			30	2.84	26.5	0.00	9.33		29.1	8.3	29	27	
4/E	70/30	5/0/25	10	4.35	56.0	0.00	12.9	0.91	67.7	7.7	45	24	67
			30	3.34	35.8	0.28	10.7		32.4	8.3	35	23	
5/E	60/40	5/0/30	10	4.23	65.1	1.61	15.4	0.92	44.4	6.7	51	25	65
			30	2.80	25.8	0.70	9.21		29.7	7.8	27	33	
6/D	40/60	30/5/30	10	6.53	95.8	0.00	14.7	0.85	50.2	7.8	37	32	77
			30	4.22	58.6	5.65	13.9		43.5	8.6	41	22	
7/D	85/15	15/5/5	10	5.46	77.3	0.00	14.2	0.89	34.7	7.6	47	25	66
			30	2.81	28.6	0.00	10.2		35.3	8.1	37	28	
8/P	20/80	40/5/20	10	3.50	55.2	33.05	15.8	1.03	28.2	8.1	43	27	65
			30	2.28	26.2	36.88	11.5		27.0	8.4	33	21	
9/E	2/98	40/5/30	10	4.96	75.5	24.58	15.2	0.60	23.1	7.9	53	23	66
			30	2.96	34.6	12.94	11.7		52.1	8.4	37	28	

E = Eumacroamphi, D = Dysmull, P = Pachiamphi – D/C: D: deciduous leaf in OL, C: coniferous remains in OL - S/R/G: S = superficial stoniness, R = rocks, G = gravel in the 0-10 layer – AWC = available water capacity estimated for the first 30 cm.

Gravel was usually frequent or common. The surface appeared scarcely rocky or rocky, with frequent or abundant stoniness. Depth was very variable, ranging from less than 50 cm to more than 100 cm. The class of biological activity was always "common", according to the soil survey manual, and dominated by lumbricids. Table 1 shows general information and analytic results of each plot. Carbon stock of the first 30 cm of the mineral soil averaged 7.24 Kg/m^2 with a standard deviation of 2.79. On the basis of the preliminary soil survey, plots 1, 5 and 8 were chosen for digging and sampling a soil profile. A synthetic description is reported in table 2. The soil typologies were Calcaric Cambisols and Cambic Calcisols, often skeletic and leptic (Fig. 1). The first and the second profile corresponded to Eumacroamphi humus while the last one to the Pachiamphi.

Table 2. *Field description and laboratory analyses of three representative soil profiles.*

Name/ Type	Horiron	Depth	Boundary	Color Munsell	Gravel	Structure	Consistence	Pore	TOC	TN	C/N	$CaCO_3$	pH
		cm	D/T	moist	V%/S	T/S/G		V%/S	%	‰		%	
Plot 1/ Calcaric Calcisol (Humic)	A1	11	C/W	10YR 3/2	15/m,f	Sbk/f/1	B	3/vf,f,m	4.03	3.98	10.1	23.1	7.8
	A2	20	C/W	10YR 4/2	15/m	Sbk/f/1	B	1/vf,f,m	2.66	2.82	9.43	24.1	8.0
	Bw	40	C/W	10YR 4/3	15/m	Sbk/f/1	B	0.5/vf	1.01	1.34	7.52	5.91	8.0
	Bk	60	A/W	10YR 4/3	15/m	Sbk/m/3	B	0.5/vf	0.45	0.91	4.96	26.3	7.7
	BCk	80	C/W	2,5Y 6/6	15/m	Abk/m/5	F		0.49	0.88	5.52	11.4	8.3
	CBk	120		10YR 5/6	15/m	Mas	F		0.33	0.83	3.93	12.5	8.4
Plot 5/ Calcaric Skeletic Cambisol (Humic)	A1	9	C/W	10YR 4/2	10/c	Sbk /c/2	B	2/m	7.24	5.16	14.0	0	5.5
	A2	28	A/W	10YR 4/3	10/c	Sbk /c/2	B	2/vf	2.29	2.84	8.07	4.28	7.2
	Bw	45	A/S	10YR 5/3	50/m,co	Sbk /vc/2	B	2/vf	1.03	1.51	6.79	8.12	8.1
	Ckr	75	C/S	2,5Y 5/3	80/s	Mas	R		0.26	0.82	3.17	7.17	8.3
	C2	95	A/S	2,5Y 6/4	80/s	Mas	R						
Plot 8/ Calcaric Leptic Cambisol (Humic)	A	18	C/W	10YR 3/2	40/m,c	Sbk /m/1	F	1/f,m	4,04	3,84	10.5	22,5	8,2
	B1	60	G/I	10YR 4/3	45/c,m	Sbk /m/1	F	1/f,m	1,95	2,13	9.15	14,6	8,1
	B2	75		10YR 4/4	absent	Sbk /m/1	F	2/f,m	0,94	1,24	7.58	1,2	8,6

Boundary. (D) ***Distinctness:*** A = abrupt, C = clear, G = gradual - **(T)** ***Topography:*** S = smooth, W = wavy, I = irregular ### **Gravel.** (V%) Gravel content % by volume - (S) Size: f = fine gravel (0.2-0.5 cm), m = medium gravel (0.5-2 cm), c = coarse gravel (2-7.6 cm), co = cobbles (7.6-25 cm), s = stones (25-60 cm) – **Structure. (T)** ***Type***: Sbk = subangular blocky, Abk = angular blocky, Mas = massive – **(S)** ***Size***: f = fine, m = medium, c = coarse, vc = very coarse – **(G)** ***Grade***: 1= very strongly developed, 2 = strongly developed, 3 = medium developed; 4 = moderately developed; 5 = low developed ### **Consistence**. B = brittle, F = firm, R = rigid **Pore. (*V%*)** Pore content - **(S)** ***Size***: vf = very fine (<0.5 mm), f = fine (0.5-1 mm), m = medium (1-2 mm), c = coarse (2-5 mm), vc = very coarse (>5 mm).

Figure 1
Profiles of the studied area. In order: Plot 1, Calcaric Calcisol (Humic). Plot 5, Calcaric Skeletic Cambisol (Humic). Plot 8, Calcaric Leptic Cambisol (Humic).

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Humus forms

Three typologies of humus were identified in the studied area. Amphi was the most common type. It is typical of area where ecological conditions are dominated by contrasting climate (dry summers and rainy falls), calcareous substrate and an artificial substitution of vegetation, with a consequent shift from rich broad-leaf litter to recalcitrant coniferous litter (Andreetta et al., 2018). Literature reports that in the Amphi humus, a slow biodegradation process (turnover of 2-7 years) is mostly caused by the endogeic and anecic earthworm activity in the organo-mineral horizon, and to arthropod and enchytraeid and epigeic earthworms in the organic horizons (Zanella et al., 2001) In the studied area, most of Amphi humus forms belonged to the Eumacroamphi typology and only one to the Pachyamphi. In Pachyamphi, the biomesostructured aggregates were more frequent, the transitions less sharp, and the OH much thicker than in Eumacroamphi (Fig. 1).

Mull system is generally associated to non-acid siliceous or calcareous parent materials, and/or easily biodegradable litter. Anecic and large endogeic earthworms are considered the main actors of a fast biodegradation that quickly moves away the litter from topsoil (turnover of about 2 years) and allows organic carbon accumulation in the A horizon (Andreetta et al., 2018). The Mull is a humus form without OH, with a thick biomacrostructured A horizon having pH ≥ 5 and which contains most of the soil OC. In this study, the Dysmull type was attributed to 2 plots where deciduous trees (turkey and ash) were dominant or abundant (about 40%). It showed a continuous OF horizons with changeable OF thickness, no OH horizon, and very sharp transition (<3mm) between organic and organo-mineral horizons. Dysmull represents the Mull class with the slowest biodegradation, and therefore represent the transition towards the Amphi type (Zanella et al., 2001) (Fig. 2).

The organic C content (g / kg of soil) in the A horizon was significantly higher in Dysmull profiles, followed by Eumacroamphi. Pachiamphi differed from the others for a much higher percentage of OC in the biomicroaggregates fraction. The lower content of OC in Amphi typologies was balanced out by thickness of this horizon. Nitrogen followed the same trend of OC (Figg.3, 4).

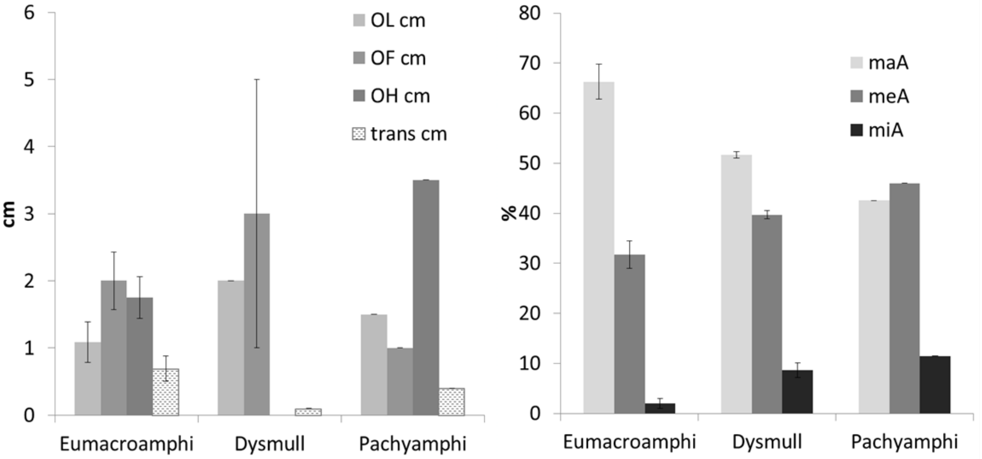

Figure 2. *Physical features of humus typologies (mean ± standard error): thickness of O horizons and percentage of aggregates in A (maA: biomacroaggregates; meA: biomesoaggregates; miA: biomicroaggregates)*

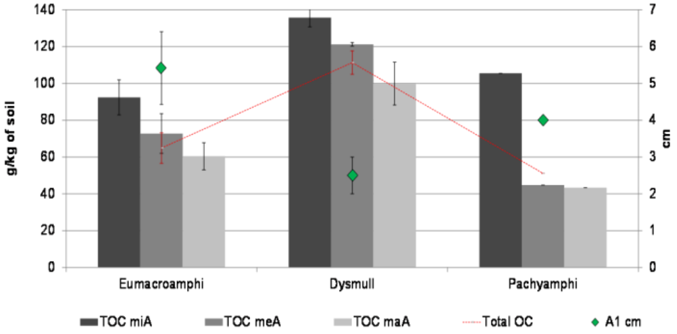

Figure 3

Organic carbon content of the aggregate fractions (columns) and of soil as a whole (line) in the A horizon; squares stand for the thickness of the analyzed A horizon (mean ± standard error).

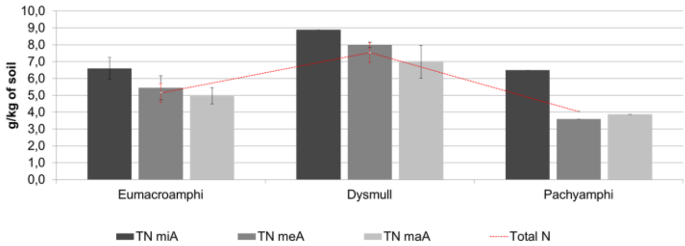

Figure 4

Grams of Nitrogen on a kg of soil in the A horizon (mean ± standard error)

C fluxes

CO_2 emissions from soil (organic and mineral layers) measured in 2016 showed an average of 32 kg ha^{-1} d^{-1}, which is comparable to other pine forests in Mediterranean environment (Matteucci et al., 2015; Romanyà et al., 2000; Rezgui et at., 2016). CO_2 emissions followed a typical seasonal trend with the highest values during summer time (Fig. 5).

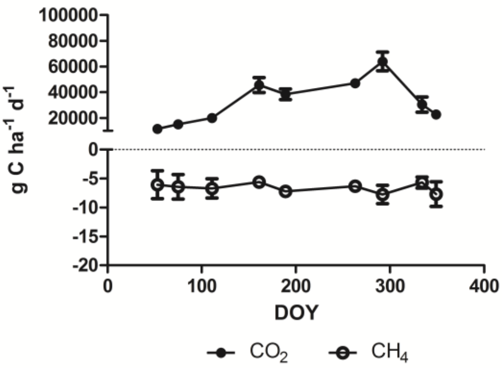

Figure 5
C fluxes from soil from February to December 2016 as mean of the nine plots and their standard error.
DOY (day of the year).

On April 2016 the largest fluxes were found from Dysmull profiles, followed by Eumacroamphi and Pachyamphi (Fig. 6, left). CH_4 showed a negative flux from atmosphere to soil, on average -6.6 g ha^{-1} d^{-1} in 2016 (Fig. 5). Whether soils are a net source or sink for CH_4 depends on the interplay between CH_4 production by methanogen and consumption by methanotroph organisms (Tate, 2015). CH_4 uptake is typical in Mediterranean and Temperate forest soils (Dong et al., 2002, Rosenkranz et al., 2006, Savi et al., 2016) where aerobic soil conditions trigger methanotrophic and atmospheric CH_4 consumption was greater than production. CH_4 uptake did not show any relationship with temperature. A larger uptake was observed in Dysmull profiles, with respect to the others (Fig. 6, right).

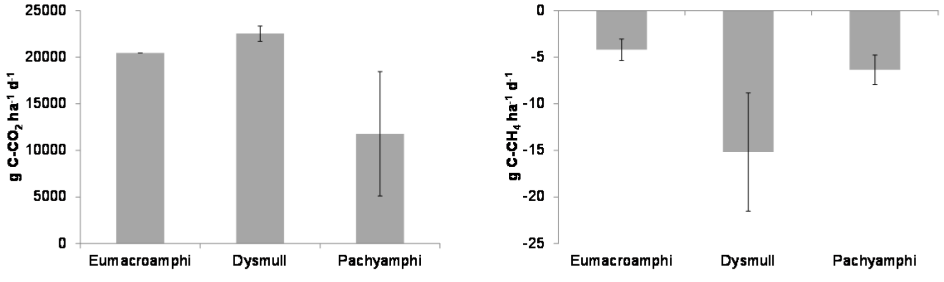

Figure 6. *Mean C fluxes from soil in April 2016 as CO_2 and CH_4 and their standard error*

Figure 7 reports differences in CH_4 uptake among three different classes of thickness of the transition layer used in terrestrial humus classification (Zanella et al., 2011.

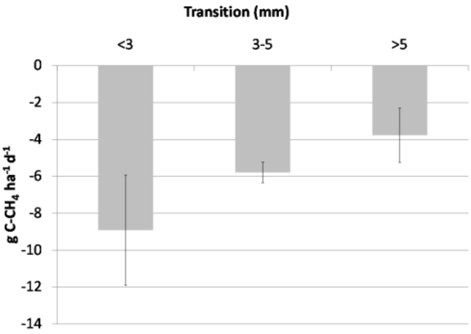

Figure 7
Histogram of mean values of CH_4 flux and their standard error for the classes of thickness of the transition layer for terrestrial humus according to Zanella et al. (2011) classification

Spearman's correlation

Our findings highlighted several correlation trends between the diagnostic humus properties, the other variables related to C fluxes, and chemical and physical soil features (table 3). The strongest correlations (p-value<0.05) regarded the thickness of O horizons and the carbon and nitrogen percentage content in the organic- mineral A horizon. The relationship was positive for OL and inverse for OH, and highlighted a different C dynamic in the two sequences.

Table 3. _Spearman's correlation of diagnostic humus terms with soil properties about C fluxes and the main physical and chemical attributes of the organo-mineral A horizon included the humus profile (**=p-level ≤0.05; *=p-level ≤0.1)._

	OL cm	OF cm	OH cm	transition mm	maA (%)	meA (%)	miA (%)
TN-A	_0,704**_	0,414	_-0,803**_	-0,451	-0,236	0,067	0,200
TOC-A	_0,729**_	0,451	_-0,642**_	-0,451	-0,515	0,418	_0,564*_
CO_2	-0,175	-0,136	-0,539	-0,384	-0,134	0,182	-0,036
CH4	-0,200	-0,186	0,533	_0,743**_	_0,584*_	-0,486	_-0,608*_
stoniness	0,354	0,186	-0,332	-0,376	_-0,646**_	_0,768**_	0,518
rocks	_0,639**_	0,332	-0,050	0,013	-0,454	0,454	0,533
gravel	0,110	_0,591*_	-0,059	0,278	0,000	0,182	0,045
BD	-0,225	_-0,242_	0,570*	0,378	0,389	-0,486	-0,255
CEC	0,025	_0,322_	-0,378	-0,087	0,219	-0,267	-0,316
pH	-0,306	_0,186_	0,768**	0,625*	0,377	-0,255	-0,170

A trend linked a decreased uptake of CH_4 with a larger thickness of OH layers (p-value = 0.11) and even more with a larger thickness of the transition between OH and A (p-value <0.05).). This was evidenced in the histogram of figure 7.

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No significant correlation was found between CO_2 emissions and the diagnostic properties of humus forms. Nevertheless, a negative trend with OH thickness was observed (p-value = 0.11).

CH_4 uptake also showed an inverse correlation with the percentage of biomacroaggregates in A and a direct correlation with the percentage of biomicroaggregates.

Diagnostic properties of humus also showed significant relationships with stoniness and rock content, where OL and OF thickness correlated positively with the abundance of rock and gravel. Stoniness significantly positively affected also meso aggregate formation but negatively macroaggregate.

Discussion

Carbon stock values of mineral horizons in the studied soils were comparable to similar woodlands of Tuscany (IFNC). The C sequestration potential of soils under a Dysmull humus type was clearly higher than the other humus forms. This trend coupled with CO_2 emission and CH_4 uptake, indicating high microbial activity. Indeed, compared with Amphi types, Dysmull resulted to be related to a major soil fauna (respiration) and aerobic metanotrophic bacteria activity.

The differences in the bio-aggregate fractions of all investigated humus types suggest that different humus forms constitute distinct biological compartments and have different effects on soil C storage. According to the known association between bio-aggregates dimension and dominant engineering organisms (Zanella et al. 2011), the highest C storage capacity should be the product of the enchiytraeid and microartrophods, main responsible for building biomicroaggregates, followed by epigeic and small endogeic earthworm. Anecic and bigger endogeic earthworms have a lower power, according to their feedings which include also large quantities of mineral matter. This trend was found by us in all the humus types and the Spearman test confirmed a positive correlation between biomicroaggrates amount and OC content. In the biomicroaggrates, the enchytraeid and microarthropod droppings contain vegetal rests and few mineral grains and are firm and hardly biodegradable (Zanella et al. 2001).

Previous studies indicated a different role of Annelida from earthworms to enchytraeids in the process of organic matter mineralization. In cultivated fields of Poland, the contribution of enchytraeids to mineralization was greater than that of earthworms (Golebiowska and Ryszkowski, 1977; Kasprzak, 1982). Several other authors stressed the Enchytraeidae significant contribute to the mineralization of organic material (e.g. Hendrix, et al., 1986, Wolters, et al., 1988, Van Vliet et al., 2004).

The positive correlation between CH_4 uptake and biomicroaggregate percentage, together with a negative correlation with the biomacroaggregates, might point to differences between enchytraeid and earthworm (anecic and endogeic) microcosms in the litter decomposition processes, specifically in the enhancement or inhibition on soil microflora. According to Wolters (1988), enchytraeids increase the inoculation capacity of the microflora by the alteration of physico-chemical conditions of the substrate, but at the same time they can reduce and select the number of soil micro-organisms by ingestion. The selective ability is not shared with the earthworms of Lumbricidae family, which do not digest microorganisms during the transit in the intestine (Zanella, 2001).

In addition, the abundance of peds in the size class <1 mm, which was probably due to a high Enchytreid and arthropodic activity, suggested stronger aerobic conditions than the pedo-environments dominated by anecic and endogeic earthworms, which move along deeper soil layers. The major content of available O_2 might explain a major CH_4 uptake.

The Spearman test also pointed to correlations between the thickness of humus layers, organic carbon content, and CH_4 and CO_2 fluxes. A larger amount of organic material in the forest floor (OL) led to a higher C stock in the soil. At the same time, the inverse correlations between OH thickness and the fauna and bacteria activity, namely CO_2 emission, and the TOC and TN content in the organo-mineral horizon, indicated that a bigger amount of humified material in the OH and transition layers reflected a slower transformation process. The transition layer OH-A showed the same trend.

The reduced activity of fauna and microflora activity, and the slower transformation of organic matter, could also be due to a higher humidity of OH compared to the less decomposed forest floor layers. A higher humidity content in OH is reported by previous studies. (Greiffenhagen et al., 2006) stated that in a German coniferous forest the soil available water (pF 1.8–4.2) ranged from 22 vol.% in OF up to 26 vol.% in OH, and that maximum values were reached in spring.

A higher thickness of OH and transition layers may also lead to i) a lower O_2 availability in the underlying soil and ii) a limitation of CH_4 diffusion from atmosphere to the soil, which in turn may reduce the methanotrophic bacteria activity responsible for CH_4 uptake. Hence our findings suggest that a soil environment with a thinner organic horizon is more suitable for the activity of aerobic methanotrophic bacteria and can favor GHG sequestration.

The lower TOC content in A, which was accompanied by thinner OH and transition layers, also caused an effect on bulk density and pH. Indeed, the negative and positive correlations between OH thickness and, respectively BD and pH, may also be due to changes in organic matter content in A.

Correlation between stoniness and rocks coverage, and humus properties, confirmed previous findings on the effect of the presence of the gravel layer in organic and organo-mineral horizons on fauna activities (Romanyà, 2000). In particular, our results suggested that the presence of rocks and stoniness allowed a bigger accumulation of leaves in the forest floor (OL) and, as a consequence, a higher content of organic carbon in the A horizon (correlation between rock amount and OH thickness at p-value <0.05; stoniness and rocks positive correlation with TOC at p-value=0.08). An effect of gravel on fauna activity was highlighted by the significant correlation between stoniness and frequency of biomacroaggregates (inverse) and biomesoaggregates (direct). Rock presence followed the same trend.

According to Poesen and Lavee (1994) rock fragments can modify the microclimate of the forest floor by intercepting water and by altering the infiltration rate, and the microclimate conditions may in turn affect the distribution and activity of soil organisms. Ferran (1997) highlighted how rock fragments may represent a limit for the circulation of soil fauna throughout the soil profile. Likewise, Fons (1995) suggested that high content of organic matter in soils with an elevated presence of rocks and stoniness might be attributed to a decrease in the transfer of particulate material induced by the presence of gravel.

In comparison with these previous findings, our results added new evidences about a differentiated effect on the pedofauna ecological groups. In particular, the gravel content seemed not to hinder the activity of the dominant engineering organisms in biomesoaggregates fraction of soil (epigeic and small endogeic earthworms, enchytraeids and arthropods). On the other side, it may represent an impediment for anecic and endogeic earthworms, which are the dominant engineering organisms for biomacroaggregates fraction.

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Conclusions

Our findings highlighted a direct relationship between the accumulation of organic material in the forest floor as organic input and the C stock in the underlying mineral soil. The C storage potential was negatively correlated with the thickness of the OH horizons. Higher thickness of the humified organic horizon may reduce the activity of the fauna and the microflora, because of a low O_2 availability.

Our study confirms the usefulness of humus forms as indicators of different ecological functionalities (De Nicola, 2014), adding new evidences of a link between biological activities and humus properties. Dysmull typology allowed a relatively larger C storage capacity of the underlying soil horizon than Amphi humus, although this ability was balanced by a thinner A horizon. A different ecological function of humus forms was also evidenced by C fluxes. Soil fauna and microflora activity, as well as the CH_4 uptake, were stronger in Dysmull, where the absence of OH horizon contributed to a more active soil ecosystem, characterized by an organic matter less resistant to decomposition processes and by a higher GHG mitigation capacity. On the bases of these findings, our study will be continued in order to evaluate the effect of different silvicultural treatments, aimed to recover the degraded forest, on the C fluxes and soil storage capacity.

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