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INFLUENCE OF *PINUS PINEA* PLANTATION ON PHYSICO-CHEMICAL AND BIOLOGICAL SOIL PROPERTIES IN QUERCUS ILEX CLIMAX AREAS IN CAMPANIA (SOUTHERN ITALY)

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Abstract

Pinus pinea plantation effects on soil physico-chemical and biological properties were investigated in two Mediterranean protected areas, where Quercus ilex woods represent the climax vegetation. Organic matter content, pH, electrical conductivity, N and S contents, available K, Mg, Mn, Cu and Fe contents, basal respiration, microbial biomass and Degens' Catabolic Response Profile were determined in Pinus pinea and Quercus ilex soils in both areas. Pinus pinea appear to affect most of the investigated soil properties; at both areas, Pinus pinea soils showed higher organic matter content and C/N ratio and lower pH than Quercus ilex soils: the availability of Mg and Fe were higher and Cu lower in pine soils. The availability of Mn and K as well as biological properties were also influenced by the tree cover but in different ways at Vesuvius and San Silvestro. Other environmental factors, such as soil mineralogic properties and seasonal effects were also important.

INTRODUCTION

Afforestation was widely practised in southern Italy, especially during the last century, and *Pinus pinea* (L.) is one of the most commonly used species due to its colonizing ability and its rapidity in producing tall woods on stony ground or very degraded soils.

Tree cover is one of the main factors affecting soil physico-chemical and biological properties, determining the quality and quantity of organic compounds that reach the soil by litter and root exudates. Moreover, tree cover influences soil microclimatic conditions, that may affect litter decomposition and soil microbial communities.

In order to investigate *Pinus pinea* plantation effects on some soil physico-chemical and biological properties we compared soils from *Pinus pinea* (L.) plots and *Quercus ilex* (L.) plots of two protected areas in Campania (southern Italy), where *Q. ilex* is the climax species and some patches had been afforested with *P. pinea*.

MATERIALS AND METHODS

Study areas

The research was carried out in two protected areas of Campania. Both sites have a typical Mediterranean climate. The first area is situated in the Vesuvius National Park, on a volcanic soil. The study area is located at “Piano delle Ginestre”, about 600 m a.s.l., where *P. pinea* was widely planted from 1912 on lavas originating during eruptions in 1822, 1858 and 1872. The climax species, *Q. ilex*, has also been widely reintroduced. We selected and marked 8 soil plots: 4 covered by *Q. ilex* and 4 by *P. pinea*. The second area is a calcareous soil from a hill behind the Royal Palace of Caserta, called “Bosco di San Silvestro”: it was originally a Bourbon hunting preserve and since 1995 it has been a WWF nature reserve. The sampling area is at the top of the hill (310 m a.s.l.), where a *Q. ilex* wood stood until the 19th century and is interrupted by some patches with 30-35 year-old pines. Six plots were selected at San Silvestro: 3 covered by *Q. ilex* and 3 by *P. pinea*.

Soil sampling

Soil samples were collected from the organic layer in April, July and December 2001, June and December 2002 from the 8 Vesuvius plots, and in February and July 2003 from the 6 San Silvestro plots. The soils were sieved (2 mm) and stored at 4℃ for biological analysis or oven dried (75 ℃) for chemical analyses.

Analytical procedure

Physico-chemical properties. Water holding capacity (WHC) was determined on undisturbed, non-sieved samples, as the weight difference between water saturated and oven dried (105 ℃) soil. Water content (WC) was determined as the weight difference between fresh sieved and oven dried (105 ℃) soil. The pH was measured in aqueous soil extract (1:10 soil:water ratio) by a Hanna Instrument pHmeter. Organic matter was determined by mass loss on ignition after 5 h at 600℃; N and S total contents by a C,N,S Analyser (Carlo Erba EA1110). Available K, Mg, Mn, Cu and Fe concentrations were measured by atomic-absorption spectrometry (SpectrAA20 Varian) in soil extracts: K and Mg were extracted by a 100 g l^{-1} of $BaCl_2$ and 22,5 ml l^{-1} of TEA, pH 8.1 solution (1:20 w:v); Mn, Cu and Fe by a 0.02 M of EDTA and 0.5 M of ammonium acetate, pH 4.65 solution (1:5 w:v).

Biological properties. The catabolic response profile (CRP) was investigated and the catabolic evenness calculated according to Degens *et al*. [1]. Two millilitres of twenty five simple organic substrates solutions, including amino acids (15 mM), carboxylic acids (100 mM) and sugars, including D-glucose, (75 mM) were added to fresh soil (1 g d.w. equivalent) in 35 ml glass vials. The pH of the solution was adjusted to 5.8-6. A soil sample with 2 ml of distilled water was included to detect the basal respiration. The vials were sealed and incubated for four hours at 25℃ in the dark. After incubation the substrate-induced respiration was determined by measuring the headspace concentration of CO_2 by gas chromatography (Fisons Instruments 8000) equipped by electron capture detector and a Porapak Q column. The catabolic evenness was calculated as $CE = 1/p_i^2$, were p_i is the respiration induced by each substrate as a proportion of the total respiration induced by all substrates.

The data from D-glucose amended soils were also used to calculate the total microbial biomass as microbial C [2,3]. The metabolic quotient (qCO_2=µg CO_2-C mg^{-1} C_{mic} h^{-1}), which indicates the degree of activity of the microbial biomass, was calculated using the basal respiration data and the microbial C.

FDA-active hyphal length was determined by the Soderstrom method [4]. The soil was suspended in 60 mM phosphate buffer pH 7.5 (1:100 ratio) and homogenised two minutes in a knife mill at top speed. 1 ml of fluoresceine diacetate (FDA) 10 µg ml-1 was added to 0.5 ml of the homogenate. The suspensions were stained for 3 min and then filtered through 0.45 µm pore size, 25 mm diameter non-fluorescent filters, embedded in non-fluorescent immersion oil and immediately observed under an epifluorescent microscope: hyphal length was estimated by the intersection method [5].

Total hyphal length was evaluated using the same method and even the same homogenate, staining the hyphae with aniline blue in 80 % lactic acid rather than in FDA; the filters were observed by bright field microscopy.

RESULTS

Physico-chemical properties. The physico-chemical properties of *Q. ilex* soils (Q) and *P. pinea* soils (P) from Vesuvius and San Silvestro are shown in table 1. At both sites *P. pinea* soils (P) showed higher organic matter (OM) content than *Q. ilex* soils (Q). OM ratio between P and L (P/L) was 1.4 and 1.2 at Vesuvius and San Silvestro, respectively. In P soils pH was lower than in Q soils at both sites, and at San Silvestro the pH was more alkaline and the gap between P and Q was even higher (1.3 units) than in Vesuvius soils (0,7 units).

Water holding capacity (tab. 1) was not significantly different between P and Q at both sites. It was about twofold higher at Vesuvius than San Silvestro. Water content differed significantly between P and Q soils at both sites. Electrical conductivity did not significantly differ between P and Q at both sites and showed values between 0.5 and 0.6.

Table 1. Organic matter (% d.w.), pH, water holding capacity (% d.w.), water content (% d.w.) and electrical conductivity (mS cm^{-1}) in *Quercus ilex* (Q) and *Pinus pinea* (P) soils at Vesuvius and San Silvestro (mean values of all samplings).

		O.M.	pH	W.H.C.°	W.C.	E.C. *
Vesuvius	P	48.6 ^{a}	4.5 ^{a}	165 ^{a}	91.0 ^{a}	0.50 ^{a}
	Q	34.3 ^{b}	5.2 ^{b}	173 ^{a}	67.5 ^{b}	0.56 ^{a}
San Silvestro	P	54.9 ^{a}	5.2 ^{a}	86.8 ^{a}	68.5 ^{a}	0.60 ^{a}
	Q	45.5 ^{b}	6.5 ^{b}	82.0 ^{a}	60.0 ^{b}	0.55 ^{a}

°determined only for the first sampling; * determined only in Dec. '01 and Jun '02 at Vesuvius, in both samplings at San Silvestro.

The concentration of some of the main nutrients and C/N ratio are shown in table 2. N content was higher and C/N ratio lower in Q than in P soils at both sites. No differences were found for S. At both sites Mg and Fe showed higher contents in P than in Q soils; the opposite held for Cu content. Mn and K contents were higher in Q than in P soils at San Silvestro but were similar at Vesuvius.

Biological properties. Biological analyses (Tab. 3) showed conspicuous differences between *Q. ilex* (Q) and *P. pinea* (P) soils. At Vesuvius basal respiration, microbial biomass and Degens' catabolic evenness showed significantly lower values in P than in Q soils. By contrast, metabolic quotient, total and FDA-active hyphal length were higher in P soils (tab. 3); only for total hyphal length was the difference not significant. At San Silvestro only total and FDA-active hyphal length were significantly different between P and Q soils: the first was lower in P and the latter in Q soils; basal respiration and microbial biomass showed a similar trend to Vesuvius but the differences were not significant. Total and FDA-active hyphal length (all data) were negatively correlated to pH ($P<0,01$ and $P<0,0001$, respectively).

Table 2. N (% d.w.), C/N, S (% d.w.), available Cu ($\mu g\ g^{-1}$ d.w.), Mg, Fe, Mn, K ($mg\ g^{-1}$ d.w.) in Quercus ilex (Q) and Pinus pinea (P) soils at Vesuvius and San Silvestro (mean values of all samplings).

		N	C/N	S	Cu *	Mg*	Fe*	Mn*	K*
Vesuvius	P	0.97 ^{a}	29 ^{a}	0.12 ^{a}	5.26 ^{a}	1.31 ^{a}	0.84 ^{a}	0.3 ^{a}	0.50 ^{a}
	Q	1.14 ^{b}	18 ^{b}	0.13 ^{a}	9.09 ^{b}	1.12 ^{b}	0.63 ^{b}	0.3 ^{a}	0.58 ^{a}
San Silvestro	P	0.86 ^{a}	17 ^{a}	0.11 ^{a}	5.33 ^{a}	0.51 ^{a}	0.42 ^{a}	0.12 ^{a}	0.30 ^{a}
	Q	0.99 ^{a}	12 ^{b}	0.12 ^{a}	7.28 ^{b}	0.44 ^{a}	0.35 ^{a}	0.26 ^{b}	0.48 ^{b}

* determined in April and December '01 at Vesuvius, in both samplings at San Silvestro.

Table 3. Basal respiration ($\mu g\ CO_2\ g^{-1}$ O.M. h^{-1}), microbial biomass-C ($\mu g\ C\ g^{-1}$ O.M.), metabolic quotient ($\mu g\ CO_2$-C mg^{-1} Cmic h^{-1}), total and FDA-active hyphal length ($m\ g^{-1}$ O.M.) and Degens catabolic evenness in Quercus ilex (Q) and Pinus pinea (P) soils at Vesuvius and San Silvestro (mean values of all samplings).

		Basal respiration *	Microbial biomass *	q CO_2 *	Tot. hyphal length °	FDA-active hyphal length°	Catabolic evenness *
Vesuvius	P	78.3 ^{a}	4.00 ^{a}	5.42 ^{a}	1880 ^{a}	1300 ^{a}	17.9 ^{a}
	Q	103 ^{b}	6.39 ^{b}	4.57 ^{b}	1688 ^{a}	525 ^{b}	19.3 ^{b}
San Silvestro	P	56.4 ^{a}	4.34 ^{a}	3.81 ^{a}	508 ^{a}	282 ^{a}	20.8 ^{a}
	Q	62.3 ^{a}	4.46 ^{a}	4.22 ^{a}	710 ^{b}	191 ^{b}	20.3 ^{a}

not determined at Vesuvius: °in April 01, *in July 01.

Catabolic evenness (Tab. 3) was lower at Vesuvius than at San Silvestro, both for P and Q soils. The catabolic response profile (Fig. 1) showed very similar patterns between P and Q soils at Vesuvius. Slight relative differences between respiration induced by some substrates are responsible for significant differences in catabolic evenness values.

By contrast, at San Silvestro the catabolic response profile showed very different patterns but very similar catabolic evenness values between P and Q soils. In both sites a seasonal effect on CRP is evident.

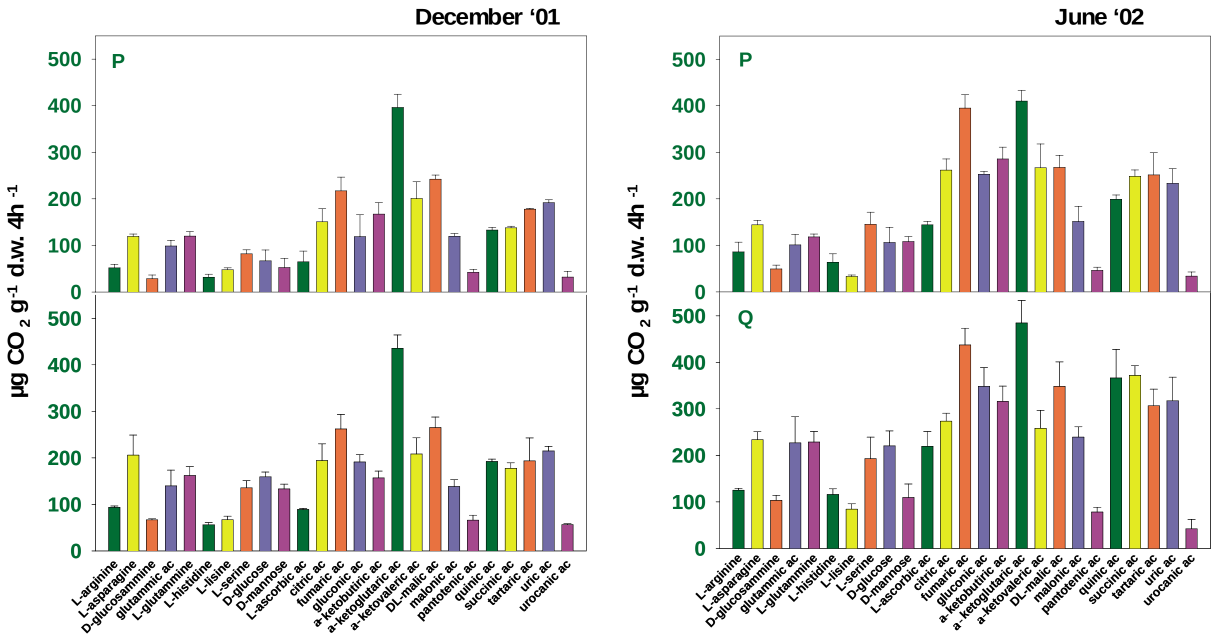

Fig. 1. Catabolic response profile of Vesuvius *Pinus pinea* (P) and *Quercus ilex* (Q) soils to 25 carbon substrates, in two samplings. Error bars represent standard error of the mean (n=4 for both P and Q soils).

DISCUSSION

The physico-chemical properties of *Q. ilex* soil (Q) and *P. pinea* soil (P) from Vesuvius and San Silvestro show an evident influence of tree vegetation, except for electrical conductivity. The higher OM content in P than in L soils is probably attributable to the slower decomposition rate of *P. pinea* than *Q. ilex* litter, due to the high phenolic compounds content in pine needles. Pinzari *et al*. [6], in central Italy, also found higher organic matter content in *P. pinea* than in *Q. ilex* soils.

Pine cover is also responsible for the lower pH in P than in Q soils at both sites, due to the acidifying power of pine needles [7].

Soil water content was influenced by a typical Mediterranean climate with dry summers and wet winters (seasonal trend not shown). No differences were found in water content between P and Q soils during the summer or periods of heavy rain, but higher values were found in P soils in wet season samplings, probably due to the higher OM content and the more compact structure of needle litter, which reduces soil water evaporation.

Electrical conductivity values indicate a similar soluble salt content in both volcanic and calcareous soils; these values are in the range (0.2-2.0 mS cm^{-1}) generally found for non-saline soils.

Pinus pinea and *Quercus ilex* cover often appear to affect soil nutrient contents in a similar way at both sites, even if differences between P and Q are not always significant. The lower N content and the higher C/N ratio in P than in Q at both sites suggest that tree cover strongly affects soil C and N contents. This is partly due to the different N content and C/N ratio between *P. pinea* and *Q. ilex* litters. In samples from Vesuvius trees, N content and C/N ratio were 0.5 % and 54.3 respectively in *P. pinea* needles [8], and 1.5% and 33.3 respectively in *Q. ilex* leaves [9]. The different tree cover did not seem to directly influence soil Mn and K contents at Vesuvius or S content at both sites.

At Vesuvius, the lower basal respiration and microbial biomass in P than in Q soils, despite the higher organic matter content, suggest that Q soils have more favourable conditions for microbial community development and activity, as confirmed by the lower qCO_2, indicative of a more efficient utilisation of carbon resources. An increase in qCO_2 is assumed as an indicator of microbial community stress [10]. At Vesuvius, the higher qCO_2 in P soils is probably attributable to the lower pH. Anderson and Domsch [10] also found higher qCO_2 in acidic soils compared to soils with more neutral pH values.

At San Silvestro, the lower pH in P than in Q soils does not correspond to a higher qCO_2. On the other hand the pH in Vesuvius P soils is even lower than in San Silvestro P soils, suggesting that a pH of 5.2 is not so low as to affect qCO_2. pH is one of the main factors affecting soil microbial community biomass, activity and structure [11, 12, 13]. The higher qCO_2 in low pH soils may reflect not only a higher maintenance energy requirement for the microbial community in response to stress, but also a shift in the bacterial fungal ratio [10]. Bååth and Anderson [12], in soils from 53 mature broad-leaved forest stands (beech and beech/oak stands), along a pH gradient, found that as pH decreased, fungal/bacterial respiration ratio (by selective inhibition of the substrate-induced respiration) decreased and bacterial/fungal biomass ratio (by PLFA tecnique) increased. Blagodatskaya and Anderson [14], in fir and beech forest soils, also found an increasing fungal/bacterial respiration ratio related to decreasing pH. Fungi are more tolerant to acidity and they are more competitive than bacteria at low pH. This is consistent with the higher FDA active hyphal length, which represents the metabolic active part of fungal biomass, and that was negatively correlated to pH.

Catabolic evenness (CE) may assume the maximum value of 25, using 25 substrates: this happens when all the 25 substrates induce the same respiratory response, i.e. if soil microbial communities oxidizes all the substrates at the same rate. Degens *et al.* [1] suggested that CE values lower than 18 are indicative of a lower quality of organic matter than soils with CE higher than 18. Moreover, Degens *et al.* [15] found that CE increased in soils along a successional gradient, showing higher values in more mature systems. Assuming these interpretations, at Vesuvius, P soils have a lower organic matter quality, despite its higher content, than Q soils. In the study area, the soil originated on lavas from recent eruptions, the last in 1944, and it is a relative young system compared to San Silvestro soils. The higher CE value at San Silvestro, without differences between P and Q may indicate a more stable soil system.

The catabolic response profile (Fig. 1) showed very similar patterns between P and Q soils at Vesuvius, even though slight relative differences between respiration induced by some substrates are responsible for significant differences in catabolic evenness (Tab 3). By contrast, at San Silvestro the catabolic response profile (Fig. 2) showed very different patterns but very similar catabolic evenness values between P and Q soils (Tab. 3). At both sites a seasonal effect on CRP is evident.

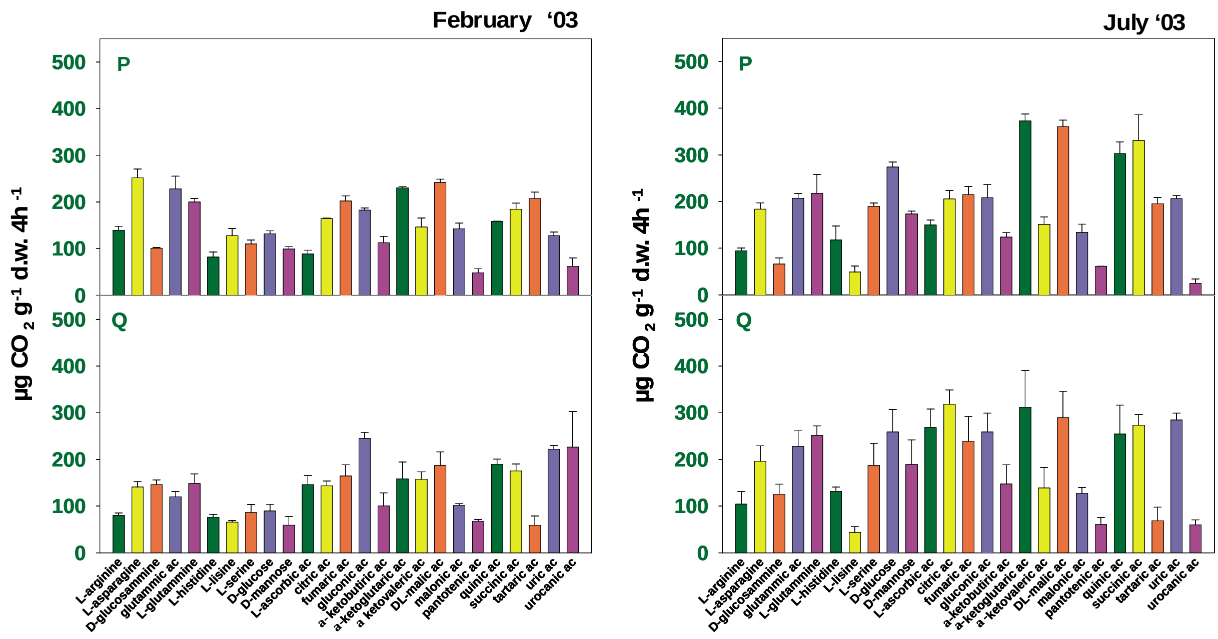

Fig. 2. Catabolic response profile of San Silvestro *Pinus pinea* (P) and *Quercus ilex* (Q) soils to 25 carbon substrates, in two samplings. Error bars represent standard error of the mean (n=3 for both P and Q soils).

5. CONCLUSIONS

These data show that *Pinus pinea* plantations induced changes in several physico-chemical and and biological properties in the two Mediterranean soils, causing an increase in organic matter and C/N ratio and a decrease in N content and pH. The availability of Mg and Fe increased and Cu decreased in pine soils.

P. pinea also affected soil available Mn and K and biological properties but in different ways at Vesuvius and San Silvestro, likely due to the different pedogenic origin of the soils. Soil microbial communities are affected by different soil chemical properties induced by the different tree cover, such as pH, quantity and quality of organic matter and C/N ratio. Their interaction with other environmental factors, such as soil mineral components and seasonal effects, also affects microbial communities in the investigated soils.

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