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Applied Soil Ecology

A novel FePO4 nanofertilizer has no acute effects on soil microbial communities and soil microbial activity

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Keywords:	Nanofertilizers; Phosphorus solubility; microbial communities; Microbial Activity; Soil Toxicity
Abstract:	We tested the effects of a novel P nanofertilizer (P-NF) on P solubility, microbial toxicity, respiration rate, enzyme activity and microbial community structure of two soils with contrasting properties in a laboratory incubation study. From the comparison with a commercial triple superphosphate (TPS), the P-NF induced lower release of soluble P, did not cause microbial toxicity, nor reduced soil respiration. Among the measured enzyme activities involved in C, N, P and S mineralization, only the protease activity was significantly inhibited by the P-NF in both the studied soils. Concerning the microbial community structure, after 1 and 7 d of incubation no significant impacts on Bacteria, Fungi and Archaea were observed. We concluded that the tested novel P-NF could be safely used.

Highlights

A P nanofertilizer (P-NF) was tested for P release and impact on soil microorganisms.

The NF showed lower P solubility, no ecotoxicity, and no reduction of soil respiration of two soils.

Among enzymes involved in C, N, P and S mineralization, only protease activity was inhibited.

No significant short-term effects on the Bacteria, Fungi and Archaea were observed.

The novel P-NF could be considered effective and ecologically safe to used.

A novel P nanofertilizer has no impacts on soil microbial communities and soil microbial activity

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Abstract

We tested the effects of a novel P nanofertilizer (P-NF) on P solubility, microbial toxicity, respiration rate, enzyme activity and microbial community structure of two soils with contrasting properties in a laboratory incubation study. From the comparison with a commercial triple superphosphate (TPS), the P-NF induced lower release of soluble P, did not cause microbial toxicity, nor reduced soil respiration. Among the measured enzyme activities involved in C, N, P and S mineralization, only the protease activity was significantly inhibited by the P-NF in both the studied soils. Concerning the microbial community structure, after 1 and 7 d of incubation no significant impacts on the Bacteria, Fungi and Archaea were observed. We concluded that the tested novel P-NF could be safely used.

Keywords: Nanofertilizers; Phosphorus solubility; Microbial Communities; Microbial Activity; Soil Toxicity

1. Introduction

Mineral fertilization is unavoidable to secure sufficient the crop production, but it is long known that it induces immediate and large release of highly soluble nutrient pools, which reduce the activity of hydrolytic enzymes involved in the nutrient mineralization (Geisseler and Scow, 2014), and alter the microbial community structure (Hallin *et al.*, 2009; Fierer *et al.*, 2012). In real farm phosphatic fertilization operations, the soil phosphate (P) pools are increased shortly (hours-days) after fertilization because mineral fertilizers release the nutrients continuously. Conversely, the nutrients demand by crops is generally low at the early crop growth stages, and quantitative uptake generally occurs weeks or months after soil fertilization. Mineral P fertilizers have a very low crop nutrient use efficiency (NUE) ranging between 10% and 25% (Huygens and Saveyn, 2018) because once added to soil as mono- and diammonium phosphate or triple superphosphate, P is released into soil as polyphosphate readily hydrolysed to orthophosphate within hours or days, due to the combined actions of water solubilization and phosphatase activity. Ortophosphate P can then be leached along the soil profile, form poorly phytoavailable precipitates with Ca or Fe- and Al- (hydr-)oxides, or be immobilized into microbial and organic pools (Baligar and Fageria, 2015).

Impacts of mineral fertilizers on soil microbial communities and environment can be reduced by slow-release fertilizers (SRFs) that release nutrients over prolonged periods rather than immediately and continuously, to better match the nutritional requirements of different crops, being the SRF grains generally coated with hydrophobic materials or enriched with enzymatic inhibitors (Robbins, 2005). However, the SRFs efficiency depends on soil properties, climatic conditions and microbial activity, and they have higher costs and environmental footprint than conventional fertilizers (Melia et al., 2017).

Nanotechnology is recognised by the European Commission as one of the six ‘Key Enabling Technologies’ that can contribute to sustainable competitiveness and growth, also in the agricultural sector (Parisi et al., 2015). Owing to their peculiar properties, NF can remain suspended into the soil solution longer than their bulk equivalents (Prasad et al., 2017; Weeks and Hettiarachchi, 2019), preventing the massive initial P release and the associated impacts on soil microbiota and environment, as compared to their bulk equivalents (Chhipa, 2019). Improved NUE and crop yields fertilized with P-NF have been reported (Liu and Lal, 2014; Marchiol et al., 2019). In a hydroponic study Sega et al. (2019) showed that a novel P-NF could be an efficient source of P and Fe for cucumber and maize plants.

Though promising, NFs must meet fundamental criteria of sustainability and ecological safety, and the fact that some nanomaterials are suspected of toxic effects on soil microorganisms raise the question on their suitability. Currently, studies on P-NFs interactions with soil microorganisms still are scarce (Dimkpa, 2014), and while Zn, Si, Fe, and Au based NFs have proven to improve the plant nutrition, uncertainties on their ecotoxicity limits their large-scale use (Holden et al., 2014). Scientific evidence shows that the impact of nanomaterials on soil microorganisms and soil enzyme activities depends on their chemical composition and exposure rates (Grün et al., 2019). We hypothesized that a novel P-NF prepared by Sega et al. (2019) could have no impacts on microbial communities and microbial activity, and prevent the immediate release of highly soluble P in different soils. Microbial toxicity of the novel P-NF was also assessed as a pre-requisite for its future safe use as soil fertilizer.

2. Materials and methods

2.1. P-NF synthesis, soil properties, and P availability

The P-NF was synthesized adding a 0.6 M K_2HPO_4 solution dropwise to 25 mL of a solution containing 0.6 M $Fe(NO_3)_3$ under continuous stirring at 600 rpm at room temperature (25 °C) as described by Sega et al. (2019). The P-NF size distribution and quantification were performed according to (Sega et al., 2019) and to Stookey (1970), respectively.

Two soils were sampled in Cesa (C) and Romola (R) areas (Tuscany, Central Italy), and their classification and main properties are reported in Table 1. Soils were sieved (< 2 mm) at field moisture, kept at 25°C for 7 d, and moistened to the 55% of water holding capacity (WHC) with deionized water (control) or the P-NF suspension equivalent to 34 mg P kg^{-1}. Samples of 50 g of each soil and treatment were incubated in airtight 1 l jars also containing a beaker with 4 ml of 0.1 M NaOH, at 25° C in the dark, and sampled after 0, 1, 4 and 7 d. All soils and treatments were prepared in triplicates for each sampling time. Samples of both C and R soils amended with triple superphosphate (TPS) at the same P level were prepared in the same way as the control and P-NF treatments and analysed only for the determination of available P. Available P was determined according to Olsen and Sommers (1982) by extraction with 0.05 M $NaHCO_3$ at pH 8.5 for 30 min, followed by P colorimetric quantification at 720 nm according to Riley and Murphy (1962).

2.2. Determination of soil respiration, soil enzyme activities and soil toxicity

Soil respiration was determined by titration of NaOH with 0.1 N HCl to quantify of the CO_2 emitted by soils (Schinner et al. 1996). Acid and alkaline phosphomonoesterase, arylsufatase β-glucosidase and protease activities were determined with the methods included in Alef and Nannipieri (1996). Microbial toxicity of P-NF was evaluated by treating the C and R soils with increasing P-NF doses equivalent to 0, 3.4, 7.1, 13.1, 26.3, 52.5, 78.8 and 105 mg P kg^{-1}. The treated soils were air-dried, and their toxicity was assessed by the BioTox™ test based on the inhibition of the *Vibrio fischeri* luminescence, soils were considered toxic when the luminescence inhibition was> 20% (Lappalainen et al. 1999).

2.3 Analysis of soil microbial community structure

Genomic DNA was extracted using the DNeasy® PowerLyzer® PowerSoil® Kit (QIAGEN) and the FastPrep-24™ according to the manufacturer instructions. Extracted DNA was amplified by PCR using the GC986f/UNI1401r (Felske et al., 1998) primers for bacterial 16S rDNA, the EF390/GCFR1 primers for fungal 18S rDNA (Vainio and Hantula, 2000) and the GC1106F/1378R primers (Watanabe et al., 2006) for the archaeal 16S rDNA, and quality and yields of PCR products were checked agarose gel with mass ladder using the Chemidoc system (Bio-Rad). The microbial community structure was analysed by the denaturing gradient gel electrophoresis (DGGE) of DNA amplicons was performed on a polyacrylamide gel with a linear denaturing gradient, with a 100% denaturant solution consisting of 40% v/v formamide and 7M urea. Gels were stained with SYBR®Gold (Invitrogen) and images were digitalized under UV light using the Chemidoc system.

2.4 Data analysis

Results of soil chemical properties and biochemical activities were analysed by one-way ANOVA followed by post hoc t-test and Tukey test using the GraphPad Prism 7 (GraphPad Software). The DGGE banding patterns were analyzed by the GelCompar II v.46 software (Applied Maths). Banding patterns were extracted as band-intensity and imported into the Past software version 3.22 (Hammer et al. 2001) for multivariate analysis. Non-metric multidimensional scaling (nMDS) was used to analyze the differences of DGGE profiles in two-dimensional space and the accuracy of the plots was determined by calculating a 2D stress value. One-way analysis of similarity (ANOSIM) was performed to determine significant differences among different treatments (Pastorelli et al. 2020). The nMDS and ANOSIM were carried out using the Bray-Curtis distance measure and with 9999 permutational tests.

3. Results and discussion

3.1. Effects of P-NF on P availability, soil respiration, soil microbial biomass and soil enzyme activities

Higher P content and availability in the control C than in control R soil could be related to its texture and neutral pH value (Kristoffersen et al., 2020) and its use for conventional maize crop rotation, and it was significantly increased by both P-NF and TSP amendments. In the R soil, the TPS significantly increased P availability as compared to P-NF after 1 and 7 d from the treatment, whereas in the C soil P availability was higher in the P-NF than in TPS treatment only after 1 d of incubation (Fig. 1a). Lower or equal P availability from P-NF as compared to TSP indicated that the novel P-NF did not rapidly dissolve in the soil solution, likely due to physico-chemical transformations such as aggregation.

Soil respiration was higher in C than R control soil, and no significant differences between respiration rates between control and P-NF treated soils after 1 and 7 d of incubation (Fig. 1b). The BioTox test showed that the P-NF caused bioluminescence inhibition ranging from 2 to the 20% did not cause toxicity for both the C and R soils, as the bioluminescence inhibition never exceed the 20% for both soils treated with any P-NF concentration. Qureshi et al. (1998) reported the ecotoxicity of a large number of chemicals detected by bacterial bioluminescence tests can be compared to that on prokaryotes, eukaryotes and humans. Globally, no inhibitory effects on soil respiration, indicating that microbial SOM decomposition activity, and lack of toxicity suggested that the tested P-NF could be considered ecologically safe, which is an important prerequisite for an eventual its future use (Gardea-Torresdey et al., 2014).

Compared to control treatment, none of the measured enzyme activities were inhibited by the soil treatment with P-NF, except for a significant reduction of the protease activity after 4 and 7 d in the C soil and 7d in R soil (Fig. 1c, d). A decrease of protease activity in soils amended with ZnO and TiO_2 nanoparticles was reported by Du et al. (2011), but studies on the impact of various nanomaterials on soil enzyme activity have resulted in contrasting evidence. For example, using a soil incubation approach similar to the present work, Eivazi et al. (2018) showed that Ag nanoparticles reduced the glycosidase and phosphatase activities, and reduction of soil microbial biomass and soil enzyme activities to various extents by different nanomaterials have been reported (*e.g.* Peyrot et al., 2013; McGee et al., 2018; Asadishad et al., 2017; You et al., 2018). In other cases, increase of different enzyme activities, including protease activity, in soil treated with NFs has been observed (*e.g.* Ge et al., 2012; Teng et al., 2018).

3.2 Impact of P-NF on soil microbial community structure

The ANOSIM and nMDS similarity analyses showed that the microbial community structure was not significantly modified by the P-NF in both the studied soils (Table 2). No significant impacts of the tested P-NF on microbial community structure differ from previous studies reporting immediate impacts of Ag-, Ti- and Au-based nanomaterials on soil microbial communities (Ge et al., 2012; Colman et al., 2013), whereas they confirmed the lack of impact of Fe-based nanomaterials. For example, Ben-Moshe et al. (2013) showed that amendment of two soils with CuO and Fe_3O_4 nanoparticles resulted in negative impacts on soil microbial communities only for CuO, not for Fe_3O_4, confirming that impact of nanomaterials on soil microbial biomass and enzyme activities depend on their chemical composition (Grün et al., 2019). No impact of the P-NF used tested in this work on bacterial, fungal and archaeal microbial communities was in agreement with the lack of microbial toxicity and no inhibition of microbial activity observed in both soils.

In conclusion, the tested P-NF incorporated into two soils with contrasting properties had no inhibitory effects on the biochemical activity, did not induce ecotoxicity nor significant changes in the microbial community structure. These results let us to hypothesize that the novel P-NF could be a suitable P fertilizer with low impact on soil microorganisms and environment, a pre-requisite for testing its efficiency towards plant mineral nutrition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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208208

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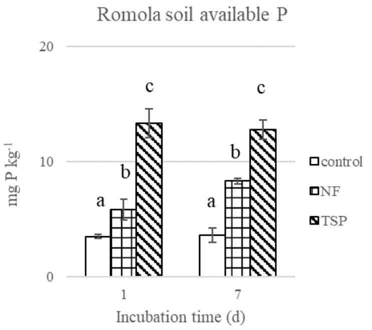
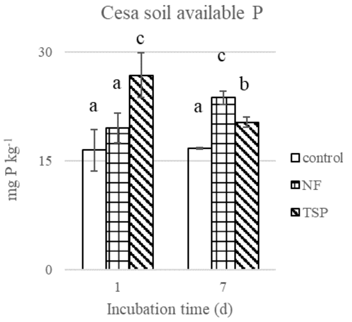
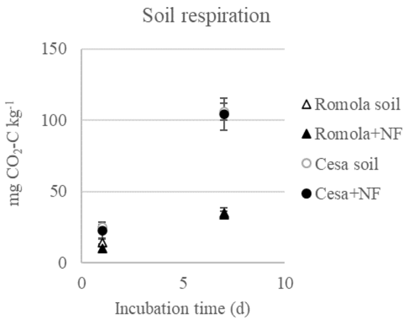
Figure captions

Fig 1. Effects of P-NF on P availability (a), soil respiration (b), soil enzyme activities (c) and protease activity (d). The error bars represent the standard deviation of the mean values. Different letters indicate significant differences. For the available P, TPS indicate the treatment with triple superphosphate.

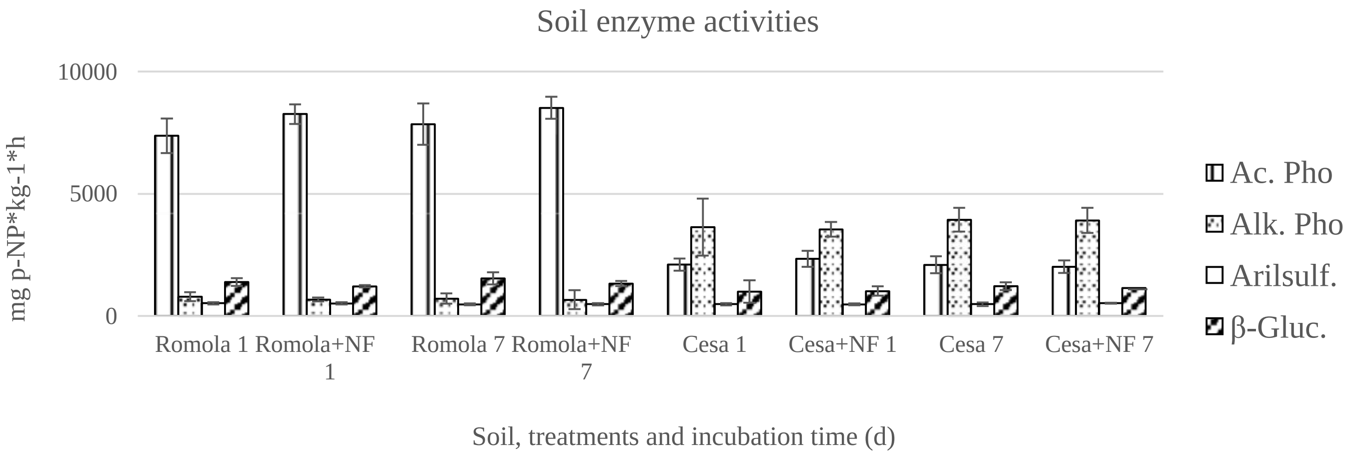
Fig. 2. Two-dimensional plots of nMDS analyses of quantitative matrixes from DGGE gel patterns for bacterial 16S rDNA, fungal 18S rDNA and archaeal 16S rDNA DGGE profiles. Samples of C and R soil treated with water (control) and with P-NF after 1 and 7 d.

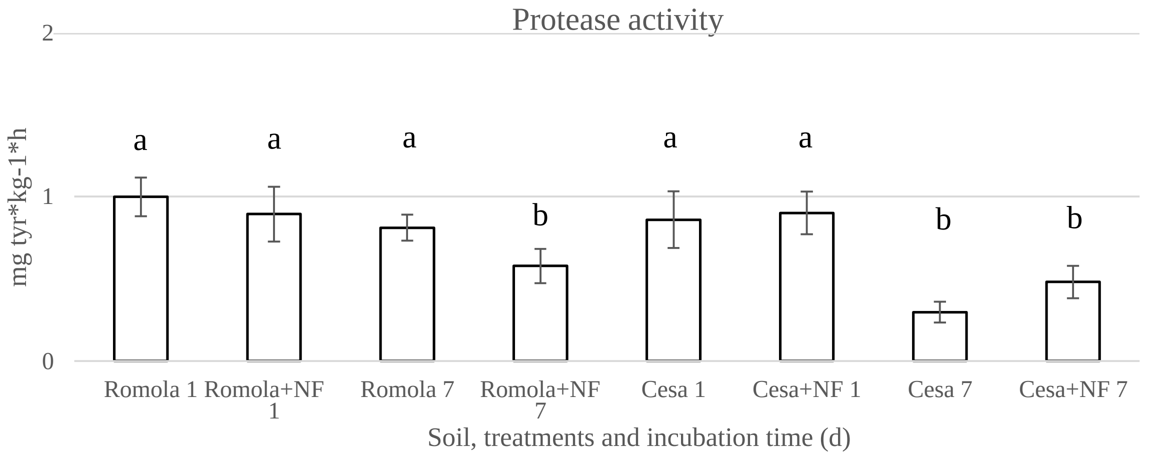

Romola soil available P
mg P kg^{-1}
20
10
0
a
b
c
a
b
c
1
7
Incubation time (d)
control
NF
TSP

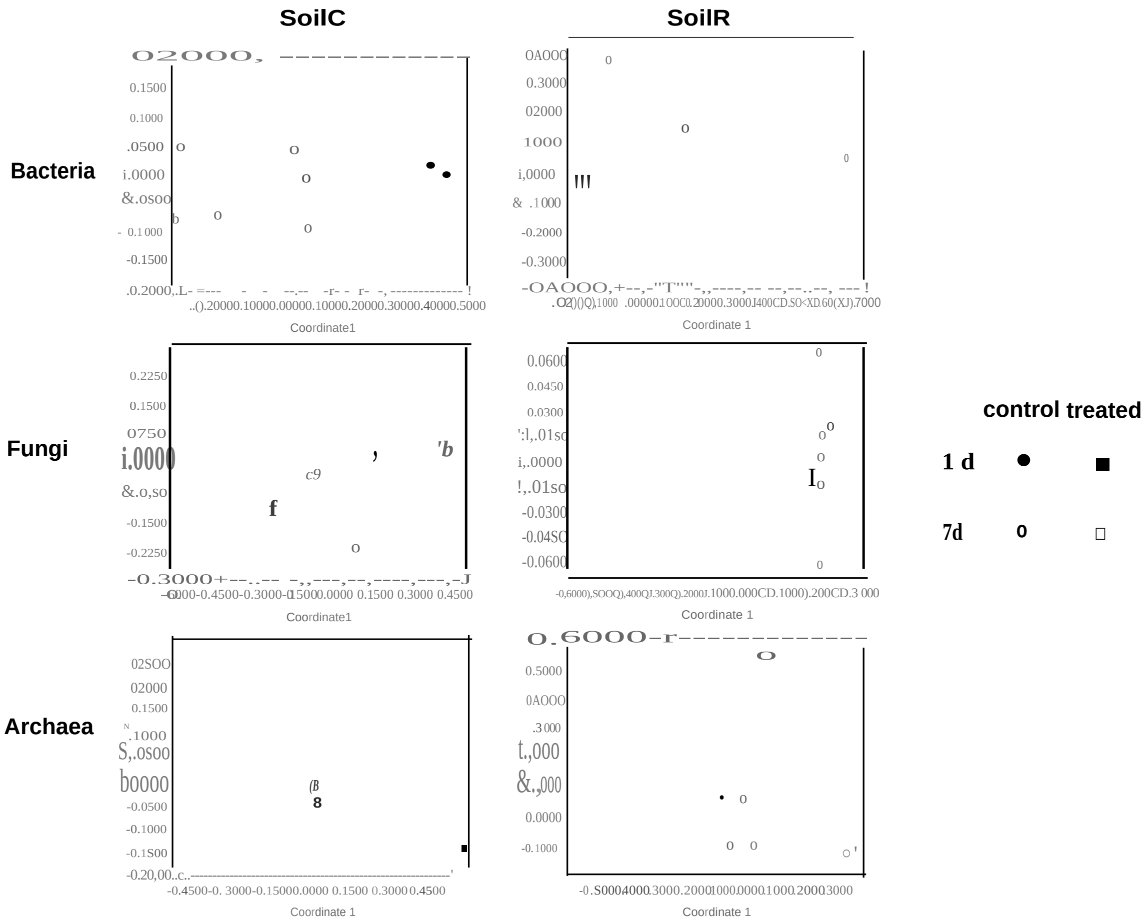

Cesa soil available P
mg P kg^{-1}
30
15
0
a
a
c
a
c
b
1
7
Incubation time (d)
control
NF
TSP


Soil respiration
mg CO$_2$-C kg^{-1}
150
100
50
0
0
5
10
Incubation time (d)
△ Romola soil
▲ Romola+NF
○ Cesa soil
● Cesa+NF

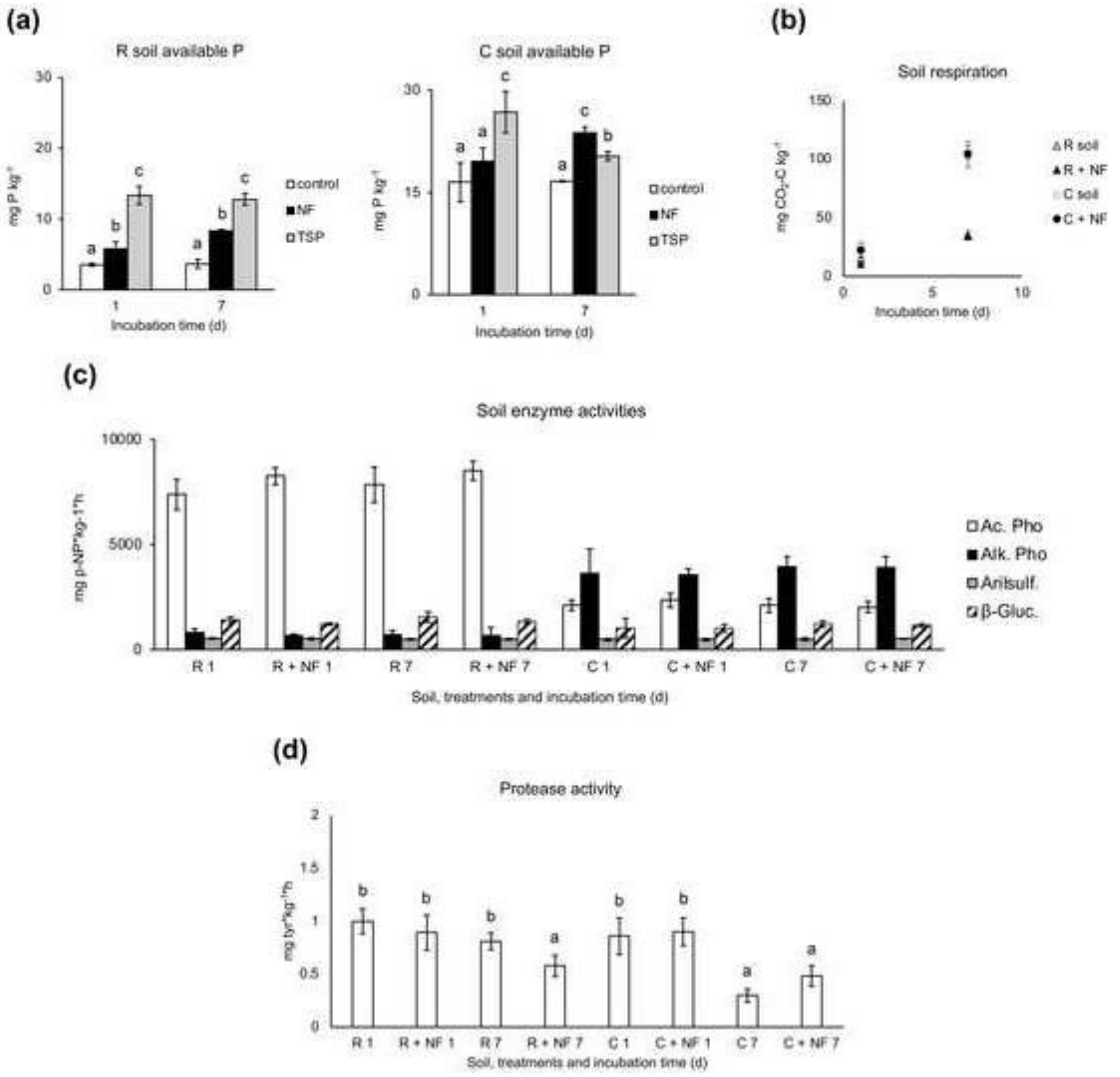
u


Soil enzyme activities
mg p-NP*kg-1*h
10000
5000
0
Romola 1
Romola+NF 1
Romola 7
Romola+NF 7
Cesa 1
Cesa+NF 1
Cesa 7
Cesa+NF 7
Soil, treatments and incubation time (d)
Ac. Pho
Alk. Pho
Arilsulf.
β-Gluc.

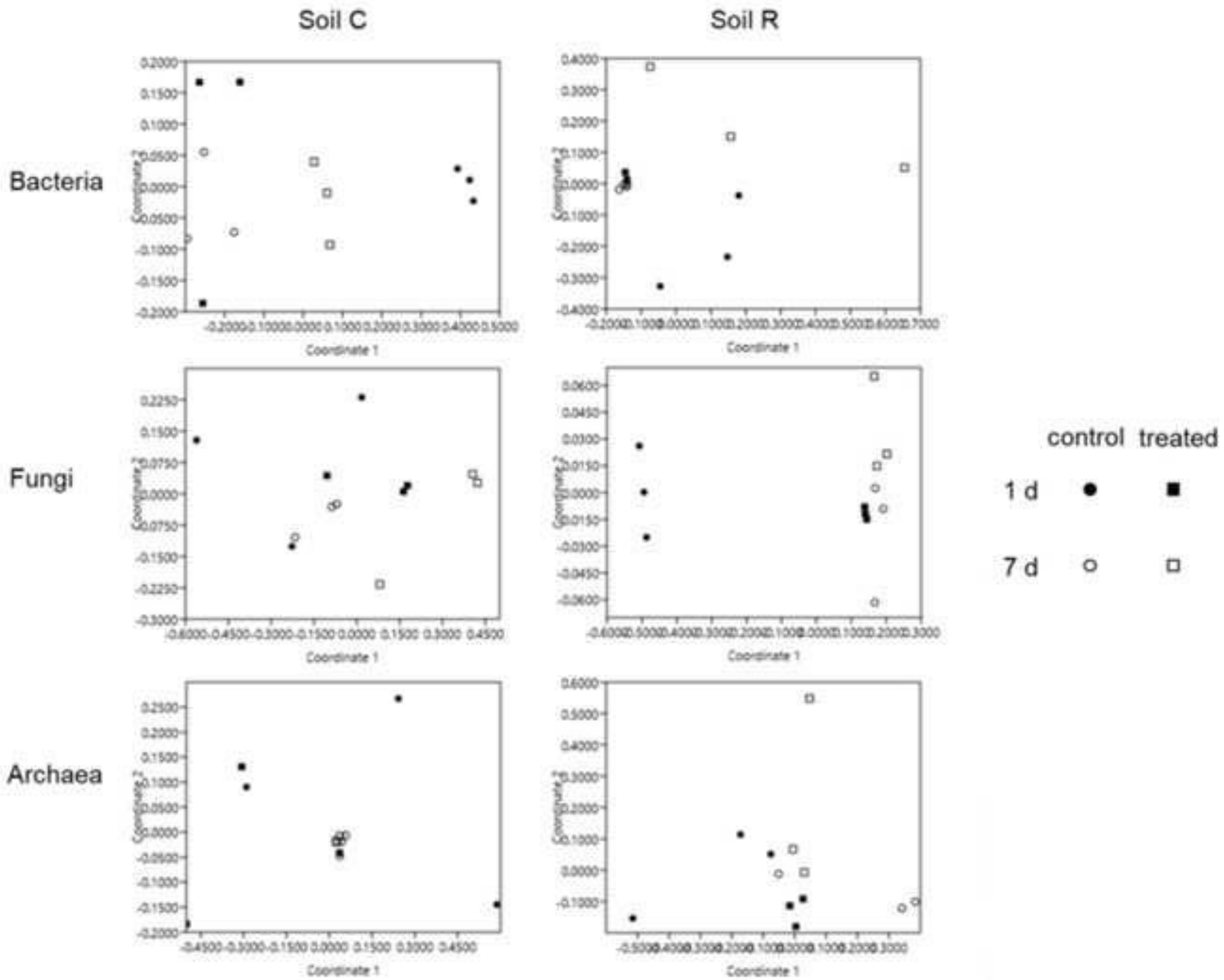

Protease activity
mg tyr*kg-1*h
2
1
0
a
a
a
b
a
a
b
b
Romola 1
Romola+NF 1
Romola 7
Romola+NF 7
Cesa 1
Cesa+NF 1
Cesa 7
Cesa+NF 7
Soil, treatments and incubation time (d)


SoilC
SoilR
Bacteria
Fungi
Archaea
Coordinate1
Coordinate 1
control
treated
1 d
7d

Figure


(a)
R soil available P
mg P kg⁻¹
30
20
10
0
a
b
c
a
b
c
1
7
Incubation time (d)
control
NF
TSP
C soil available P
mg P kg⁻¹
30
15
0
a
a
c
a
c
b
1
7
Incubation time (d)
control
NF
TSP
(b)
Soil respiration
mg CO₂-C kg⁻¹
150
100
50
0
0
5
10
Incubation time (d)
R soil
R + NF
C soil
C + NF
(c)
Soil enzyme activities
mg p-NP*kg-1*h
10000
5000
0
R 1
R + NF 1
R 7
R + NF 7
C 1
C + NF 1
C 7
C + NF 7
Soil, treatments and incubation time (d)
Ac. Pho
Alk. Pho
Arilsulf.
β-Gluc.
(d)
Protease activity
mg tyr*kg⁻¹*h
2
1.5
1
0.5
0
b
b
b
a
b
b
a
a
R 1
R + NF 1
R 7
R + NF 7
C 1
C + NF 1
C 7
C + NF 7
Soil, treatments and incubation time (d)

Figure


Soil C
Soil R
Bacteria
Fungi
Archaea
Coordinate 1
Coordinate 2
control
treated
1 d
7 d

Tables

Table 1. Main physico-chemical proprieties of C and R soils.

Soil	Classification (WRB 2006)	Sand g kg^{-1}	Silt g kg^{-1}	Clay g kg^{-1}	pH	TOC g kg^{-1}	N_{TOT} g kg^{-1}	P_{TOT} g kg^{-1}
C	Sandy clay loam Eutric Cambisol	19.8	65.2	15	7.1	0.78	0.10	6.45
R	Sandy loam Eutric Cambisol	74.4	18.1	7.5	5.4	1.71	0.11	2.22

Table 2. ANOSIM analyses of quantitative matrixes from DGGE gel patterns for bacterial 16S rDNA, fungal 18S rDNA and archaeal 16S rDNA DGGE profiles. Samples of C and R soil treated with water (control) and with P-NF after 1 and 7 d.

		Microbial groups		
C soil		Bacteria	Fungi	Archaea
Factor: Time	R	0.7963	0	0.2222
	P	0.0103	0.4754	0.0394
Factor: Treatment	R	0.94444	0.42593	0.2037
	P	0.0121	0.0314	0.1262
R soil				
Factor: Time	R	0.6204	0.83333	0.5
	P	0.0031	0.0108	0.0101
Factor: Treatment	R	0.72222	0.59259	0.38889
	P	0.0027	0.0097	0.0198