Soil Biology & Biochemistry 42 (2010) 1596–1606

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Microbial performance under increasing nitrogen availability in a Mediterranean forest soil

D. Dalmonech [a,*], A. Lagomarsino [b], M.C. Moscatelli [b], T. Chiti [a], R. Valentini [a]

[a] Department of Forest Environment and Resources, University of Tuscia, Via S. Camillo De Lellis, Viterbo 01100, Italy
[b] Department of Agrobiology and Agrochemistry, University of Tuscia, Via S. Camillo De Lellis, Viterbo 01100, Italy

A R T I C L E I N F O

Article history:
Received 22 October 2009
Received in revised form
27 May 2010
Accepted 29 May 2010
Available online 10 June 2010

Keywords:
N availability
Microbial community
C-turnover
Enzymes
CLPP
Functional diversity

A B S T R A C T

In forest ecosystems, the external nitrogen (N) inputs mainly involve wet and dry depositions that potentially alter inorganic N availability in the soil and carbon (C) turnover. This study assesses the effect of a slow increase of inorganic N availability on microbial community activity and functionality in a Mediterranean forest soil. A four-month incubation experiment was performed with soil collected from the organic layer of a forest site and fertilized with a solution of ammonium nitrate. The fertilizer was supplied at an equivalent of 0, 10, 25, 50 and 75 kg N ha^{-1} (0, 0.3, 0.7, 1.3 and 2 mg N g^{-1} for control N0 and treatments N1, N2, N3 and N4, respectively). The incubation was carried out under optimal conditions, with the addition of the nutritive solution in small aliquots once a week to mimic the phenomenon of N deposition. In order to isolate the effect of N, the pH of the NH_4NO_3 solutions was adjusted to soil pH, and phosphorus was added in order to prevent any nutrient limitation effect. Inorganic N, C-mineralization, the activity of one oxidative enzyme (o-diphenol oxidase) and 8 hydrolitic enzymes (α-glucosidase, β-glucosidase, *N*-acetyl-β-D-glucosaminidase, cellulase, leucine amino-peptidase, acid phosphatase, butyric esterase and β-xylosidase) and the community level physiological profile (CLPP) were measured and analyzed during the whole incubation and at the end of the experiment as a proxy for microbial decomposition activity. In the first month, the highest N availability (N4) repressed the microbial respiration activity but stimulated microbial enzymatic activity, suggesting a change of C-pathways from spilling to enzymes and biomass investment. The treatments N1, N2 and N3 had no effect in the same period. Throughout the incubation, a general stress condition affected all the treated soils. As a consequence, treated soils exhibited higher respiration rates than the control. This was accompanied by a loss of functional diversity and an end-detected decline in biomass C. Although at the end of incubation most of the soil features showed a clear correlation with the inorganic N pool, the organic C content was strongly affected by different patterns of microbial activity during the experiment: the highest N treatment (N4) showed a lower C loss than the N3 treatment. Overall, the experiment showed how inorganic N availability can potentially alter the C cycle in a Mediterranean forest soil. The effect is non linear, depending on microbial community dynamics, on the community's ability to adapt given the time scale of the process, and on N supply amount. Our study also revealed a common pattern in the short-term response to N addition in other, similar ecosystems with different climatic conditions.

1. Introduction

Soil inorganic nitrogen (N) availability in temperate regions can be altered by several factors, such as increased mineralization due to rising temperature (Rustad et al., 2001; Melillo et al., 2002), changed hydrological regime, and increased N external input through N deposition (Galloway et al., 2003) and fertilizers. In undisturbed forest ecosystems, N inputs mainly involve N deposition, although biological N-fixation may also be a contributing factor. Increased rates of organic matter decomposition (through the consequent release of N) provide an almost continuous input to the mineral soil pool. However, N deposition appears to be the most important factor boosting mineral N (N_{in}) availability. In European forests, for example, wet and dry N depositions are reported to range between 10 and 75 kg ha^{-1} y^{-1} (Gundersen et al., 1998; Macdonald et al., 2002), with an increasing trend.

Forest soil inorganic N availability controls processes such as litter and soil organic matter (SOM) decomposition, and plant productivity (Berg and Matzner, 1997; Magnani et al., 2007). Hence, increasing N inputs can alter the carbon (C) balance in these natural

* Corresponding author. Tel.: +39 0761 357412; fax: +39 0761 357389.
E-mail address: d.dalmonech@unitus.it (D. Dalmonech).

0038-0717/$ – see front matter
doi:10.1016/j.soilbio.2010.05.034

ecosystems, affecting the potential of forests to mitigate the increasing CO_2 concentration in the atmosphere (Hungate et al., 2003). At present, the magnitude and the direction of soil response to rising incoming N are still the subject of debate. Soil reacts according to several factors, such as the type of ecosystem, litter quality and the microbial community (Waldrop et al., 2004; Sinsabaugh et al., 2005, 2008; Allison et al., 2009), the rate of fertilization, and N demand (Knorr et al., 2005). Although no clear and predictable pattern emerges, a review by Reay et al. (2008) suggested a general trend of forest soils becoming C-sinks in response to increased N_{in} availability.

Soil C dynamics are affected by microorganisms through their involvement in organic matter decomposition (Nannipieri et al., 2003; Caldwell, 2005), and therefore any change in N_{in} could affect soil C sequestration. Several mechanisms have been proposed to explain the microbial response to N_{in} increase, but none is definitively supported by the existing evidence.

Over the last 20 years, soil incubation experiments with N fertilization have been performed on organic/mineral soils and litter (Berg and Matzner, 1997; Tirukkumaran and Perkinson, 2000; Vestgarden, 2001) across climatic gradients and differing soil N status under natural conditions (Michel and Matzner, 2003). Most of these studies, however, were conducted in the nutrient-limited temperate forests of northern Europe; little is currently known about microbial activity within the C-cycle in forest soils under a Mediterranean climate. Recently, the focus has shifted towards litter decomposition processes in Mediterranean forests (Fioretto et al., 2005; Alarcón-Gutiérrez et al., 2008), but knowledge about the response of microorganisms to a change of inorganic N_{in} in these soils is still exploratory. Although Mediterranean ecosystems are usually characterized by a small soil organic C-pool compared to northern European forests, Mediterranean areas are very susceptible to external disturbances of a climatic and anthropogenic nature. It is a priority, then, to gain a better understanding of whether the C cycle may be altered due to increased N_{in} availability.

The present work describes a 4-month incubation experiment investigating i) the impact of a simulated slow increase of N_{in} on the microbial performance in a Mediterranean forest soil; ii) how the microbial response to N_{in} availability might consequentially affect soil C-turnover; and iii) if and how this response depends on fertilizer amount and incubation time.

Our approach aims to offer a new perspective in the study of microbial responses to N availability in soil. Laboratory conditions allowed a tighter control on C and N dynamics. Thanks to this setting, we obtained two independent measures of C-mineralization and C-substrate utilization via community level physiological profile (CLPP) analysis and enzyme analysis, which relates microbial activity with the community's functional diversity. Moreover, the laboratory approach excluded most limiting factors such as moisture, temperature, and nutrient availability, thus isolating the effect of N_{in}. Finally, the innovation with respect to other, similar incubation experiments is the supply of N_{in} in small aliquots throughout the experiment, simulating a gradual increase of soil N concentration. In contrast, most other studies supplied N_{in} in a single dose at the beginning of incubation (Södeström et al., 1983; Tirukkumaran and Perkinson, 2000; Michel and Matzner, 2003; Alarcón-Gutiérrez et al., 2008) or added it throughout the period but avoided N_{in} accumulation (Magill and Aber, 2000; Vestgarden, 2001).

2. Materials and methods

2.1. Site characteristics and sampling

Soil samples were collected at the Roccarespampani forest site (Viterbo, Central Italy, 42.40 Lat, 11.93 Long). The forest is a 19-year-old coppice of *Quercus cerris* L. characterized by a Mediterranean climate with an average annual temperature of 14 °C and an average annual rainfall of 755 mm. It is subject to coppice with a rotation cycle of 20 years, and the soil, classified as *Chromic Luvisol*, shows an average organic layer of 4 cm. More detailed information on the coppice characteristics is reported in Tedeschi et al. (2006).

Composite samples were collected at three different points, corresponding to the three profiles opened at the site, covering a total area of 150 m^2. To investigate the effect of increasing N_{in} availability in the late stage of organic matter decomposition, the soil samples were collected from the organic layer, including soil mixed with decomposed plant residues (Tedeschi et al., 2006). Still recognizable plant material was removed by hand-picking. Soil was subsequently sieved at 2 mm and stored at −4 °C until termination of the experimental set-up. Subsamples were taken to determine the soil properties reported in Table 1.

2.2. Experimental design

Soil samples were pooled together in order to minimize any interference due to the heterogeneity of the site. Samples were then preconditioned at 28 °C and 60% of the water holding capacity (WHC) 24 hours before starting the incubation. Different amounts of nutrient solution were added by dissolving inorganic N in the form of ammonium-nitrate salt (NH_4NO_3) in deionized water. Samples were treated with the equivalent of 0, 10, 25, 50 and 75 kg N ha^{-1} (hereafter named N0, the control, and N1, N2, N3, N4, the treatments) corresponding to a final dose of 0, 0.3, 0.7, 1.3 and 2 mg N g^{-1} fresh soil values, which are in line with the literature. Control soil samples were treated with an equal amount of deionized water. We simulated a slow and a more realistic increase of soil N status by supplying the total dose of NH_4NO_3–N in small constant aliquots during the incubation period. An additional advantage of the slow increasing level of N was to limit the initial osmotic shock for microorganisms and allow the microbial community to deal with a slow change in the nutrient and chemical state of the soil. A single aliquot was manually added once a week; the amount of nutrient solution added to soil samples was chosen depending on the average water loss of the sample in one week at 28 °C.

Since the aim of the experiment was to investigate the effect of N_{in} only, the NH_4NO_3 solutions were prepared adding KH_2PO_4 in ratio N:P = 5:1 in order to satisfy the stoichiometrical microbial need, thus preventing an a priori nutrient limitation effect (Allison and Vitousek, 2005). The N-solutions were corrected to soil pH with NaOH 0.1 M to avoid any substrate-induced pH effect on microbial activity. All samples, prepared in three replicates, were incubated for 4 months in a thermostatic chamber at 28 °C and at 60% WHC, allowing microorganisms to work at optimal conditions. The weight of each sample was recorded at the beginning of the treatment and soil moisture was adjusted twice a week with deionized water in order to maintain soil moisture between 55% and 60% WHC.

Table 1
Initial soil features of the investigated soil samples (fresh soil). Standard deviation ($n = 3$) in parenthesis.

TOC	%	15 (0.11)
TN	%	1.15 (0.053)
C/N		13 (0.51)
pH		6.7 (0.05)
Biomass C	μg BC g^{-1}	1818.8 (198.5)
N–NH_4	μg N–NH_4 g^{-1}	20.15 (4.7)
N–NO_3	μg N–NO_3 g^{-1}	14.21 (1.17)
Mineral P	μg P g^{-1}	4.80 (0.48)