**Biogeosciences
Discussions**

The strength of the biotic compartment to retain nitrogen additions prevents nitrogen losses from a Mediterranean maquis

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Abstract

Nitrogen (N) is one of the nutrients most limiting to ecosystem productivity. However, N availability is increasing globally, which may affect ecosystem functions and stability. To understand the role of each ecosystem compartment in the cycling of increased N,
5 we studied the initial response of a nutrient-poor ecosystem, a Mediterranean maquis, to increased N. N availability (dose and forms) was modified by three N additions along the year (spring, summer and middle autumn/winter). Soil inorganic N pools (nitrate in particular) strongly reflected the N additions in autumn, almost matching the total N added along the three additions. *Cistus ladanifer*, the dominant plant species, re-
10 sponded to the increased N (cover and N concentration in leaves and litter) and given that leaf shedding occurs in the summer, the importance of this N pool returning to the soil through litter decomposition on the total soil inorganic N in autumn was investigated. Data suggest that living plants and litter have a crucial role in preventing N losses from Mediterranean maquis. This is the first integrated field study on how
15 European Mediterranean ecosystems retain increased N of different forms and doses, however longer-term studies are needed to explore the generality of this study's observations.

1 Introduction

Temporal patterns of inorganic nitrogen (N) turnover and plant growth can influence
20 important aspects of plant community and ecosystem dynamics; e.g. N losses, plant productivity, community composition, changes in N and/or phosphorus (P) limitation, decomposition rates and N niche complementarity (Augustine and McNaughton, 2004). Anthropogenic activities have led to global N enrichment (Galloway et al., 2008), threatening ecosystems at local, regional and global scales (Cassman et al., 2003), since N
25 availability has become decoupled from demand. Nutrient availability is a key determinant of ecosystem function and stability (Bobbink et al., 1998; Phoenix et al., 2006).

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When N availability exceeds an ecosystem's N retention capacity, a shift from a closed internal N cycle to an open and leaky cycle occurs, with the N in excess being leached and/or emitted from the ecosystem (de Schrijver et al., 2008). Most research on the effects of N enrichment on ecosystems has focused on temperate synchronous systems (availability of resources coincides with plant growth) from northern Europe and America (Bobbink et al., 2010; Phoenix et al., 2006). Therefore not much is known about asynchronous ecosystems (availability of distinct resources and plant growth do not occur at the same time) such as those in Mediterranean regions (Jackson et al., 1988). Mediterranean-type ecosystems occur worldwide and are characterized by the Mediterranean climate: hot dry summers, and mild wet winters (Rivas-Martínez et al., 2004). The few studies of the N retention capacity of Mediterranean-type ecosystems have been made in California (e.g. Holub and Lajtha, 2004), while, as far as we are aware, none have been made of the Mediterranean Basin.

We hypothesize that the mild temperatures and non-limiting water availabilities, which result in intense biological activity during the Mediterranean spring, coincide with periods of low soil inorganic N pools. During this period, most additional N is likely to be taken up by plants and microorganisms, and retained within the ecosystem's biotic compartment. However, during the dry summer, a significant proportion of the N will return to the soil through leaf shedding. In autumn, when water no longer limits biological activity, N returns to the inorganic form through decomposition processes. As a result, soil inorganic N pools are expected to be the highest in autumn. For this reason we studied soil inorganic N over time, plant community and the N stored in plants and litter in a Mediterranean maquis in response to N additions. Our objective was to evaluate the short-term N retention capacity of a Mediterranean maquis and understand which ecosystem compartments are involved in the cycling of the increased N in such an asynchronous ecosystem. Specifically, we examined relationships between the N-driven response of the N concentrations in the soil, leaves and litter through two consecutive springs (2007 and 2008).

2 Material and methods

2.1 Study site

The study site (38°29' N–9°01' W) is in Serra da Arrábida in the Arrábida Natural Park, south of Lisbon, Portugal (a Natura 2000 site – PTCON0010 Arrábida/Espichel). It is located in a sub-humid thermomediterranean bioclimatic domain (Rivas-Martínez et al., 2004). According to records (1971–2000 – Instituto Nacional de Meteorologia e Geofísica), mean annual precipitation is 730 mm; mean maximum temperature, 27.8 °C (August); and mean minimum temperature, 8.1 °C (January). Estimated background N deposition is 5.2 kg ha^{-1} yr^{-1} (2.9 kg NO$_x$ + 2.3 kg NH$_y$ – http://webdab.emep.int/Unified_Model_Results/AN/).

The study site is located on a southeast-facing slope (5 %) at 130 m a.s.l. that is protected from public access and has not been affected by human activity in recent years. Soil is skeletal (15 cm depth) so that true profiles cannot be discerned and soil density is 1.3 g cm^{-3}. Silt predominates in the soil (50 %), while sand and clay contents are 32 % and 18 %, respectively (silt-sand-loam texture). The vegetation consists of a dense maquis (Eunis class F5.2 – Mediterranean maquis), which developed after a fire event (summer 2003) four years before the first N addition. The vegetation is dominated by summer semi-deciduous species which exhibit leaf dimorphism, shedding an important fraction of their leaves and twigs in the summer, and whose development is coupled to water availability in the upper soil layers (Correia and Catarino, 1994; Cruz et al., 2008). At the moment, the dominant plant species is *Cistus ladanifer* L. (Dias et al., 2011). Other abundant plant species include *Erica scoparia* L., *Calluna vulgaris* (L.) Hull, *Genista triacanthos* Brot., *Ulex densus* Welw. ex Webb, *Dittrichia viscosa* L., and *Myrtus communis* L. Herbaceous species comprise ≈10 % of the total plant cover, of which many are annual plants.

2.2 Experimental design and fertilization schedule

The criteria for choosing the N doses and forms used in the experiment were: (a) N doses lower than the N deposition reported for other areas in Mediterranean-type ecosystems ($145 \, \text{kg} \, \text{N} \, \text{ha}^{-1} \, \text{yr}^{-1}$ – Fenn et al., 2003; Meixner and Fenn, 2004) but high enough to establish "worst case" scenarios of N enrichment in this type of habitat; and (b) mimic the most likely N pollution scenarios in the experimental area, i.e. combined inputs from urban/industrial sites and agricultural and predominantly agricultural sources. Therefore, N availability was modified by the addition of 40 and $80 \, \text{kg} \, \text{N} \, \text{ha}^{-1} \, \text{yr}^{-1}$ in the form of NH_4NO_3 (doses designated 40AN and 80AN) and $40 \, \text{kg} \, \text{N} \, \text{ha}^{-1} \, \text{yr}^{-1}$ as a 1:1 mixture of $N\text{-}NH_4Cl$ and $N\text{-}(NH_4)_2SO_4$ (designated 40A). Control plots were not fertilized. Beginning in January 2007, the dry N salts were homogenously added, by hand, in three equal applications over a year: spring, summer and middle autumn/winter. The N granules dissolved rapidly (1–7 days depending on the N addition period). N additions were scheduled so that no precipitation was predicted for at least one week after the N addition. N additions took place in January, April and August 2007; and January 2008, always following the corresponding soil sampling. Each treatment was replicated three times, 3 plots each of $400 \, \text{m}^2$. In order to restrict boundary effects and dilution processes, all measurements, analyses and sample collection were performed within an internal $100 \, \text{m}^2$ square. To prevent N "contamination" through runoff from N-plots, the experimental plots were distributed in three rows along the 5 % slope, with the controls being located in the top row.

2.3 Soil and plant sampling and plant assessment

Soil was sampled from the four corners and the centre of the internal $100 \, \text{m}^2$ square of each plot. Soil samples (2 cm diameter and 15 cm depth) were removed, sieved (2 mm) and stored at $4 \, ^{\circ}\text{C}$ until analysis. Sampling took place in May, August and October 2007; and February and April 2008, corresponding to the distinct seasons. Individual soil samples (five per plot) were used to determine soil pH, moisture and concentrations

of nitrate ($N\text{-}NO_3^-$), ammonium ($N\text{-}NH_4^+$) and inorganic N. Bulk soil samples (equal mixtures of the five soil samples from each experimental plot) collected in May 2007 and April 2008 were used for soil characterization (Table 1, see below for methods).

The vegetation at the study site was assessed within one $5 \times 5 \, \text{m}$ square per plot (within the internal $100 \, \text{m}^2$) during spring (June 2007, May 2008). *C. ladanifer* plant cover was calculated from the total projected crown area (calculated from two perpendicular diameters, assuming elliptical shape).

C. ladanifer's leaves were sampled in the spring. Samples consisted of twigs containing approximately 4–5 pairs of fully expanded leaves. One twig per plant was sampled from ten random *C. ladanifer* plants within the internal $100 \, \text{m}^2$ square. The leaves from the ten twigs were bulked to form one composite sample per plot. Litter was sampled near to each of the five soil sampling points in each plot. Litter sampling took place in the summer. Only leaf litter from *C. ladanifer* was analyzed.

2.4 Chemical analysis

From each individual soil sample (five per plot and per sampling time), 10 g fwt of soil were used to gravimetrically determine soil water content (Kern EG300 3M). Five g (fwt) of soil were used to prepare soil water extracts ($1:10 - w/v$). Soil extracts were shaken (Cassel Agitator, 600 rpm) for one hour at room temperature, centrifuged (Eppendorf Centrifuge 5403) at 5000 g for 20 min at $4 \, ^{\circ}\text{C}$. The supernatant was collected and analyzed colorimetrically (spectrophotometer Tecan Spectra Rainbow A-5082) for nitrate (Matsumura and Witjaksono, 1999), ammonium (Cruz and Martins-Loução, 2000), and for soil pH (Crison micro pH 2002). Soil inorganic N was the sum of the water extracted $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$, and was expressed as µg N per g of dry soil. The bulk soil samples used for determining organic matter and concentrations of total N and extractable P, potassium (K) and magnesium (Mg) were dried at $35 \, ^{\circ}\text{C}$ until constant dry weight. Organic matter was determined according to ISO standard 10694 by loss on ignition overnight at $600 \, ^{\circ}\text{C}$ (Nabertherm L3/11/C6). Analysis of total N was carried out according to ISO standard 13878 by dry combustion using an elemental analyzer

(Leco CNS). Extractable phosphorus and potassium were quantified by a modification of the Egner-Riehm method using plasma emission spectrophotometer with an optical detector (ICP-OES), following extraction using ammonium lactate 0.1 M and acetic acid 0.4 M, pH 3.65–3.75. Mg was extracted with ammonium acetate 1 M, pH 7 and quantified by atomic absorption spectrophotometry with flame atomization. Leaf and leaf litter samples were dried at 60 °C, grounded (MM 2000) and analyzed for total N and carbon (C – the same procedure as for soil samples).

2.5 Calculations

Changes in the cover of *Cistus ladanifer* over time (t_0, t_1) were calculated as follows:

$$\text{Changes over time (\%)} = \frac{(\text{Parameter}_1 - \text{Parameter}_0)}{(\text{Parameter}_1 + \text{Parameter}_0)/2} \cdot 100$$

Changes in the treatments (m_n) in relation to the control (M_c) were calculated as follows:

$$\text{Changes in relation to the control } (\mu g\, g^{-1}) = m_n - M_C$$

Where "m" corresponds to each individual value (e.g. concentration of N as soil inorganic N and litter N); "M_C" corresponds to the mean value of the control for each parameter; and "n" corresponds to the distinct experimental plots.

Transformation of the applied N doses into soil inorganic N concentrations ($\mu g\, N\, g^{-1}$ dwt) was based on the following soil characteristics: 15 cm depth and 1.3 g cm^3 of density, resulting in ~2000 t of soil per ha. Therefore, the addition of 40 and 80 kg N per ha corresponded to doses of 20 and 40 $\mu g\, N\, g^{-1}$, respectively. The transformation of the applied N doses into soil inorganic N concentrations will be referred to as "total N added", while the concentration of soil inorganic N in relation to the control determined in autumn will be referred to as "measured N".

The possible contributions to the soil inorganic N concentration determined in autumn were identified as: inorganic N present in the soil in the previous season – [N]

summer; the subsequent N addition – [N] addition; and the extra N present in the litter (in relation to the control) which was shed during the summer – [N] litter. The [N] addition and the [N] litter were also transformed into soil inorganic N in the soil ($\mu g\, g^{-1}$ soil dwt). The effects of the N additions on the three contributors were then compared to the control. The changes of litter N concentration in relation to the control were estimated assuming an annual litter production of 2.3 t ha^{-1} in a Mediterranean maquis (Schultz, 2002). Then we calculated the sum of the soil inorganic N determined in the summer, the summer N application and the N contained in the litter ("calculated N").

2.6 Statistics

Summary statistics of soil and plant parameters were compared for the different treatments. The repeated measures test (General Linear Model) was applied to assess the existence of significant interactions between time and treatment for soil and plant parameters and differences per treatment in leaf and litter parameters were analyzed by a one-way ANOVA. In both cases, these were followed by a Bonferroni test ($p < 0.05$ for all comparisons except for the changes in *C. ladanifer* cover $p < 0.1$), or by a Games-Howell test whenever homogeneity of variances was not confirmed by a Levene's test. Differences between "total N added" and "measured N" and between "measured N" and the "calculated N" were analyzed by the t-student test ($p < 0.01$). In all cases, preliminary analyses were performed to ensure that there was no violation of the assumptions regarding the tests' application. SPSS software, version 19.0, was used for all tests.

4 Discussion

4.1 Short-term response of a Mediterranean soil to N enrichment

Soil fertility of the Mediterranean Basin is considered to be moderate to high within the Mediterranean-type ecosystems (Cowling et al., 1996). However, soils at the experimental site (Table 1) had lower N concentration than those reported in other studies in the Mediterranean Basin (e.g. Gallardo et al., 2000; Ferran et al., 2005; Sardans et al., 2008; Rutigliano et al., 2009). The level of extractable P was low in comparison with other Mediterranean Basin soils (Dumontet et al., 1996; Carreira et al., 1997; Ferran et al., 2005; Saura-Mas et al., 2009) but comparable with those from Australian and South African Mediterranean-type ecosystems (Milewski, 1983; Mitchell et al., 1984; Hobbs, 1995). Soil organic matter values were within the range observed in other Mediterranean Basin soils (Peñuelas et al., 1999; Gallardo et al., 2000; Cruz et al., 2008) but so low that these soils are at risk of desertification due to soil erosion (López-Bermúdez and García-Gómez, 2006). Soil acidification (Table 1) is among the most commonly reported effects of N enrichment and may result from cation loss, aluminum release and nitrification. Although soils at the experimental site were slightly acidic, nitrification occurred since NO_3^- was the predominant N source present in the soil, even in the treatments where N was added as NH_4^+ (Fig. 1). However, soil acidification tends to be less intense in soils of the Mediterranean Basin than of other ecosystems due to the high cation exchange capacity of these soils and of the alkaline sands transported from North Africa by winds (Ochoa-Hueso et al., 2011).

Many studies of soil inorganic N availability sampled soils in spring, when water and temperature do not limit biological activities (Fig. 1a), and therefore conditions are optimal for plant growth, and concluded that the values obtained could be used as indicators of the N availability in the system. However, comparison of soil inorganic N concentrations determined in the two consecutive springs (2007 and 2008) showed no differences between treatments or between years (Fig. 1b). This may imply that the N added to the system was either lost (NO_3^- leaching, runoff, NH_4^+ volatilization,

denitrification) or incorporated in the biota (microbial community and vegetation). If most of the added N had been lost from the system, the peak of soil inorganic N observed in autumn (Fig. 1b) should only reflect (if at all) the previous N addition (summer 2007). However, the observed increase in soil inorganic N in autumn was related not only with the previous N addition, but also with the "total N added" at that sampling time (Table 2). The mild temperatures and the adequate water availability (Fig. 1a) may have stimulated the decomposition of the large litter input derived from the leaf shedding of summer semi deciduous plants (Correia, 1988) and the death of annual plants in the summer. In fact, N mineralization has been shown to peak in early autumn in Mediterranean maquis soils (Rutigliano et al., 2009; Simões et al., 2009). Thus, the reduced biological activity in the summer that allowed inorganic N to accumulate, combined with the summer N fertilization and the high autumn N mineralization (Rutigliano et al., 2009), all appear to contribute to the peak of soil inorganic N in autumn (Fig. 4). The inorganic N concentrations detected in the soil in autumn suggest that the ecosystem was more efficient in retaining $N-NH_4NO_3$ than solely $N-NH_4^+$. This shows that although undetectable as soil inorganic N along the year, most of the added N was used and conserved in the system as would be expected in a nutrient limited ecosystem (Craine, 2009). These results clearly show that early autumn is the best time of the year to measure soil inorganic N as an indicator of the N fluxes between the biotic and abiotic compartments. The N incorporation into the biotic compartments is in agreement with the fast responses of the ecosystem structure and function to increased N availability (Dias et al., 2011). If a large fraction of the N added to the system is moving through the biotic and abiotic compartments of the ecosystem, then large impacts on ecosystem processes can be expected (e.g. Emmett, 2007; Bobbink et al., 2010).

4.2 Short-term response of the plant community to N additions

The cover of *C. ladanifer* (a slow growing conservative species) (Dias et al., 2011) increased under all treatments except 80AN (Fig. 2). In nutrient limited ecosystems, N additions tend to induce changes in the plant community promoting a decrease in