



N-driven changes in a plant community affect leaf-litter traits and may delay organic matter decomposition in a Mediterranean maquis

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ABSTRACT

Organic matter (OM) decomposition is typically controlled by climate, soil properties, litter quality and soil microorganisms. Availability of nitrogen (N) also influences decomposition, but its effects on decomposition are controversial and most studies have only addressed decomposition of individual plant species grown under high N availability. We experimentally manipulated the dose of available N in a Mediterranean Basin maquis in south-western Europe, with low ambient N deposition ($5.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and low soil N content (0.1%). N availability was modified by the addition of 40 and 80 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ as NH_4NO_3 . Control plots were not fertilized. After 2.5 years of N additions, we accounted for the integrated effects of N enrichment on litter decomposability taking into consideration the N-driven changes in the whole plant community (changes in plant species composition and litter quality). We collected soil from the no N addition treatment (control) and three types of leaf-litter (from three N addition treatments – 0, 40 and 80 $\text{kg N ha}^{-1} \text{ yr}^{-1}$) from the N-manipulation field experiment and performed a microcosms controlled decomposition study. Distinct leaf-litter traits were quantified (N and lignin concentration and C/N and lignin/N ratios) and related with decomposition and soil microbial biomass and activity. The leaf-litter consisted mostly of leaves from summer semi-deciduous shrubs, but relative to the control (no N addition), the treatment receiving 80 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ had twice the amount of evergreen sclerophyll leaf-litter and higher lignin and N concentrations giving lower C/N and lignin/N ratios. As a result, OM decomposition in the microcosms containing 80 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ litter was slower (with concomitant reduction in soil microbial biomass and activity) than in those containing 40 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ litter. At the ecosystem level, N-driven changes in plant community altered leaf-litter traits (e.g. increased litter lignin and N content and decreased lignin/N ratio), which were powerful determinants of litter decomposition rates under controlled conditions. The results suggest that increasing N availability in this nutrient poor Mediterranean maquis may select species with litter traits that could delay decomposition and increase soil OM accumulation.

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1. Introduction

The balance between vegetation inputs and organic matter (OM) decomposition determines the size of the soil OM pools (Baer et al., 2010) and controls nutrient cycling in terrestrial ecosystems (Knorr et al., 2005). In turn, the rate of litter decomposition is

controlled by climate, soil properties, litter composition (Fioretto et al., 1998, 2001; Alarcón-Gutiérrez et al., 2008; Austin and Ballaré, 2010) and soil microbial community (Fioretto et al., 2001). However, nitrogen (N) availability can also influence decomposition and nutrient-cycling dynamics (Schimel and Bennett, 2004; Knorr et al., 2005; Liu et al., 2010), with potential consequences for OM decomposition and accumulation.

Increased N availability can change plant community litter traits directly and/or indirectly. Depending on the plant species and/or community, N enrichment can directly increase litter N

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concentration and decrease the C/N ratio of whole plant communities (e.g. Californian coastal sage scrub and chaparral – [Vourlitis et al., 2009](#)) and/or individual plant species (e.g. *Cistus ladanifer* from Mediterranean Basin maquis – [Dias et al., 2012](#)), making litter more readily decomposable. N enrichment is a powerful indirect driver of plant diversity changes ([Sala et al., 2000](#)), with alterations in plant traits (e.g. species-specific N and lignin concentrations) strongly influencing litter inputs and decomposition rates ([Cornwell et al., 2008](#)).

[Knorr et al. \(2005\)](#) conducted a meta-analysis of empirical studies to examine the effects of N enrichment on litter decomposition, concluding that N enrichment could increase, decrease or have no effect on litter decomposition depending on fertilization rate, site-specific ambient N-deposition level, and litter quality. However, none of the analysed studies had considered the effect of increased N availability on local plant biodiversity and consequently on leaf-litter traits.

In this work we intended to assess the effects of increased N availability on leaf-litter decomposability at the plant community level, in particular, in the semi-natural Mediterranean Basin ecosystems, where N deposition is predicted to increase three fold by 2050 ([Galloway et al., 2004](#); [Phoenix et al., 2006](#)). The dynamics of litter decomposition need to be understood in order to inform management of these biodiversity hotspots ([Phoenix et al., 2006](#)). Recently, [Incerti et al. \(2011\)](#) developed a process-based model of litter decomposition for Mediterranean ecosystems, but did not account for the effect of the initial N content of litter. The few studies made on the effects of increased N on decomposition of Mediterranean litter ([Sirulnik et al., 2007](#); [Alarcón-Gutiérrez et al., 2008](#); [Kazakou et al., 2009](#)) were not conclusive ([Ochoa-Hueso et al., 2011](#)) and did not consider N-driven changes at the whole community level. In order to elucidate the integrated effects of N-driven changes on plant litter on decomposition, the present study was performed in a N-poor Mediterranean ecosystem very responsive to N availability ([Dias et al., 2011a, 2012](#)), whose vegetation may be grouped into two main plant functional types: summer semi-deciduous and evergreen-sclerophylls. Each group has been characterized on the basis of its phenology ([Correia, 1988](#)), water relations, carbon exchange properties ([Werner et al., 1999](#)), soil surface characteristics ([Cruz et al., 2008](#)), N use ([Dias et al., 2011b](#)) and strategies for regeneration after fire ([Trabaud, 1981](#); [Keeley, 1986](#); [Clemente et al., 2005](#)).

Our objective was to focus on the biotic processes of decomposition ([Austin and Ballaré, 2010](#)), so the leaf-litter had to be crushed (1 mm). Under field conditions this would cause its loss due to wind and/or rain, thus overestimating decomposition, so we setup a litter decomposition experiment under controlled conditions (microcosms). Acknowledging the importance of drying-rewetting events, especially in Mediterranean ecosystems ([Fierer and Schimel, 2002](#)), and that in maquis soils decomposition peaks in autumn ([Rutigliano et al., 2009](#); [Simões et al., 2009](#)), we mimicked litter decomposition after a long dry period similar to the Mediterranean summer. To exclude the effect of N-driven changes in soil microbial community, we used only one type of soil (Control) and three types of leaf-litter (from three N addition treatments – 0, 40 and 80 kg ammonium nitrate-N ha⁻¹ yr⁻¹). Soil and litters were collected from an ongoing N-manipulation field study in a Mediterranean Basin maquis where increased N concentration (and decreased C/N ratio) of the dominant plant species ([Dias et al., 2012](#)) and changes in plant community ([Dias et al., 2011a](#)) had already been observed. Our working hypothesis was based on the differences between leaf and litter traits of summer semi-deciduous and evergreen sclerophylls ([Schlesinger and Mavis, 1981](#); [Correia and Catarino, 1994](#); [Fioretto et al., 2005](#)): the N-driven changes in the relative contribution of each group to whole

community litter may result in alterations of whole community litter traits (e.g. N and lignin content, C/N and lignin/N ratios) and may not be proportional to the availability of N, partially explaining the controversial results concerning the effect of N availability on decomposition rates.

2. Materials and methods

2.1. Study site and N-manipulation experimental design

The study site (38°29'N, 9°01'W) is 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). Background N deposition is estimated to be 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 N-addition field experiment study is located on a south-east-facing slope (5%) at 130 m a.s.l. Soil is a lithosol (15 cm depth) with a bulk density of 1.3 g cm⁻³, with a silt-sand-loam texture (50% silt, 32% sand and 18% clay). The vegetation consists of a dense maquis (Eunis class F5.2 – Mediterranean maquis), with closed vegetation, composed mainly of shrubs with few annuals and some geophytes, that developed after a fire event (summer 2003) four years before the first experimental N addition. It is dominated by summer semi-deciduous species (≥50% cover in control plots), exhibiting leaf dimorphism, shedding an important fraction of leaves and twigs in the summer ([Cruz et al., 2008](#)). Summer semi-deciduous species are common in open and disturbed stands, but they are progressively eliminated under the canopies of evergreen sclerophylls characteristic of late successional stages ([Correia and Catarino, 1994](#)). Sclerophyll leaves are long-lived, consistent, hard and coriaceous ([Mooney et al., 1983](#)). At the time of sampling for this study (August 2009), the dominant plant species was *C. ladanifer* L. (*Cistaceae*). Other abundant plant species included *Myrtus communis* L. (*Myrtaceae*), *Quercus coccifera* L. (*Fagaceae*), *Pistacea lentiscus* L. (*Anacardiaceae*), *Erica scoparia* L. (*Ericaceae*), *Calluna vulgaris* (L.) Hull (*Ericaceae*), *Genista triacanthos* Brot. (*Fabaceae*), *Ulex densus* Welw. ex Webb (*Fabaceae*) and *Ditrichia viscosa* L. (*Asteraceae*). Herbaceous species comprised ≈ 10% of the total plant cover, of which many were annual species ([Dias et al., 2011a](#)).

The N additions used in the field experiment were lower than the N deposition reported for other areas in Mediterranean-type ecosystems (145 kg N ha⁻¹ 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. The form of N added was chosen to mimic the most likely N pollution scenario in the experimental area, i.e., combined inputs from urban/industrial sites (oxidized N) and agricultural (reduced N). Accordingly, N availability was modified by addition of 40 and 80 kg N ha⁻¹ yr⁻¹ in the form of NH₄NO₃, (designated 40N and 80N, respectively). Control plots were maintained without N-addition. Beginning in January 2007, the dry fertiliser was spread evenly, by hand, in three equal applications per year: winter, spring and summer. Each treatment was replicated with 3 plots each of 400 m². In order to restrict boundary effects and dilution processes, all measurements, analyses and sample collection were performed within an internal 100 m² square. To prevent N 'contamination' through runoff from the N-plots, the experimental plots were distributed in three rows along the 5% slope, with the controls located in the top row (the experimental setup is further described in [Dias et al., 2011a, 2012](#)).

2.2. Soil and leaf-litter sampling

Soil and litter were collected in August 2009 (after two and half years of N additions) prior to the summer N addition. At the time, the 40N treatment plots had received a cumulative N addition of ~ 107 and the 80N plots, ~ 213 kg N ha $^{-1}$. Community leaf-litter (mixture of litter derived from the different standing species) was collected from each experimental plot from all the N addition treatments (control – Control litter; 40N – 40N litter; and 80N – 80N litter). Intact leaf-litter from the uppermost layer (0–2 cm above the soil surface) was collected by hand from four points per experimental plot, mixed (4 samples from each plot), air-dried (35 °C for six weeks) and hand-sorted according to plant species. The plant species were sorted according to functional groups (summer semi-deciduous, evergreen sclerophylls and graminoids) and the relative abundance of the leaf-litter from each of the three functional groups was calculated based on its contribution to the weight of the whole leaf-litter sample from each field N treatment (Fig. 1). Leaf-litter from the distinct functional groups was mixed again and its chemistry (total C, N, phosphorus, potassium, calcium, magnesium, sulphur, sodium and manganese – Table 2) was determined on the composite samples. Three subsamples of litter per treatment were stored at -20 °C for lignin analysis (Table 1 and Supplementary data Table 1). Soil was collected (50 cm diameter and 15 cm depth) from all control plots, sieved (2 mm), bulked (4 samples from each plot \times 3 control plots) and air-dried (35 °C for six weeks to mimic summer drought).

2.3. Litter decomposition microcosm experimental design

Although litter decomposition comprises photodegradation and biotic degradation (Austin and Ballaré, 2010), we focused solely on the biotic component. Therefore each microcosm consisted of 6 g dwt of roughly crushed litter (mortar and pestle, 1 mm), incorporated within 500 g of dry soil from the control field plots (no N addition), as described by Madritch and Hunter (2003). The microcosm (soil + litter) treatments were: i) soil only (No litter microcosm); ii) soil + litter from Control plots (Control microcosm); iii) soil + litter from the field addition of 40 kg N ha $^{-1}$ yr $^{-1}$ (40N microcosm); and iv) soil + litter from the field addition of 80 kg N ha $^{-1}$ yr $^{-1}$ (80N microcosm). The microcosms treatments were analysed for pH (H $_2$ O), OM, total C and N, extractable P, potassium and magnesium (Table 3) and incubated under controlled conditions with a photoperiod of 16 h day: 8 h night at 25 °C and 40–60% of water-filled pore space (determined

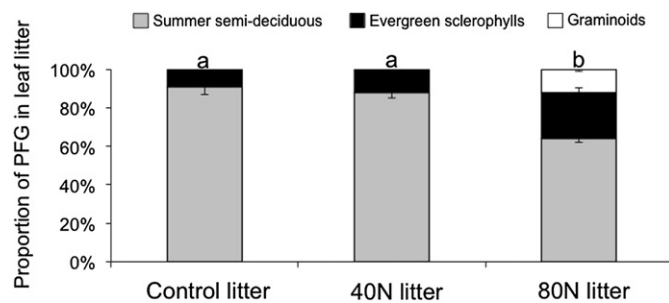


Fig. 1. Effect of the field N additions (no N addition, 40N and 80N) on the composition of the respective litters (Control, 40N and 80N litters) collected at the study site in summer 2009 (after two and half years of N additions). Stacked bars represent the proportion of the three plant functional groups (PFG – summer semi-deciduous, evergreen sclerophylls and graminoids) in the leaf-litter produced by the standing plant community ($n = 3$ microcosms) \pm SE. Different letters refer to statistically significant differences between treatments (ANOVA $p < 0.05$ followed by a Bonferroni test).

Table 1

Ratios and concentration of lignin and aromaticity of the different types of litter (Control, 40N and 80N litters) derived from the ^{13}C CPMAS NMR spectra (Supplementary data Table 1). Litters were collected at the study site in August 2009 (after two and half years of N additions). Different letters refer to statistically significant differences between treatments (ANOVA $p < 0.05$ followed by a Bonferroni test) and are shown in bold. Values represent the mean ($n = 3$) \pm SE.

	Control litter	40N litter	80N litter
Ratios			
Alkyl C/O-alkyl C	0.3 \pm 0.0	0.3 \pm 0.0	0.4 \pm 0.0
Alkyl C/Carboxyl C	3.6 \pm 0.3	3.3 \pm 0.3	3.3 \pm 0.3
O-alkyl C/Aromatic C	4.8 \pm 0.3^a	4.6 \pm 0.3^a	3.6 \pm 0.3^b
Concentration (%)			
Lignin	25.7 \pm 0.9^b	26.1 \pm 1.3^b	31.1 \pm 1.7^a
Aromaticity	20.3 \pm 0.7^b	20.6 \pm 0.9^b	22.0 \pm 1.1^a

gravimetrically) for three months (Madritch and Hunter, 2003). Given the dimension of the litter particles and the proportion of soil to litter in the microcosms, litter was not distinguishable from the soil at the beginning or the end of the incubation period. Each microcosm treatment was replicated 3 times and microcosms were randomly distributed in the incubator.

2.4. Chemical analysis

Frozen (-20 °C) bulk leaf-litter (collected from the N-manipulation field experiment) and pre-incubation soil microcosm samples were analysed for their lignin concentration and aromaticity (Table 1 and Supplementary data Table 1) by ^{13}C Cross-Polarization Magic-Angle Spinning Nuclear Magnetic Resonance (^{13}C CPMAS NMR), using a Bruker Avance III 400 MHz spectrometer and a commercial two-channel, 4-mm Bruker probe head. Dried samples were ground (particle size 0.3 mm) and analysed as described by Alarcón-Gutiérrez et al. (2009). Based on previous studies (see Alarcón-Gutiérrez et al., 2009) it was possible to characterize the signals of the ^{13}C CPMAS NMR spectra of the distinct litter types. Using the deconvolution software DmFIT (Massiot et al., 2002), the aromaticity index (AI) was calculated as $\text{AI} = 100 [\text{A} (110\text{--}160 \text{ ppm}) / \text{A} (0\text{--}160 \text{ ppm})]$ (Lorenz et al., 2006), while lignin concentration was estimated using the following equation established by Haw et al. (1984): % lignin = $(100\%) (183 / 9.92) (I'_{\text{lig}} / (183 / 9.92) I'_{\text{lig}} + (162 / 6) I'_{\text{carb}})$.

Dried litter samples were analysed for total C, N, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), sodium (Na) and manganese (Mn) as described by Fioretto et al. (2001, 2003). At the beginning of the experiment, soil microcosms, i.e.,

Table 2

Effect of the treatments (no N addition, 40N and 80N) on nutrient concentrations in the respective litters (Control, 40N and 80N litters) collected at the study site in August 2009 (after two and half years of N additions). Different letters refer to statistically significant differences between treatments (ANOVA $p < 0.05$ followed by a Bonferroni test) and are shown in bold. Values represent the mean ($n = 3$ experimental plots per treatment) \pm SE.

	Control litter	40N litter	80N litter
C (mg g $^{-1}$)	453.7 \pm 8.9	455.3 \pm 10.3	443.7 \pm 7.0
N (mg g $^{-1}$)	5.7 \pm 0.3^b	8.0 \pm 0.6^a	10 \pm 0.6^a
P (mg g $^{-1}$)	0.2 \pm 0.0	0.2 \pm 0.0	0.3 \pm 0.0
K (mg g $^{-1}$)	1.0 \pm 0.1^b	1.4 \pm 0.1^a	1.3 \pm 0.1^a
Ca (mg g $^{-1}$)	10.8 \pm 1.6	12.4 \pm 1.0	13.6 \pm 1.1
Mg (mg g $^{-1}$)	1.1 \pm 0.1	1.3 \pm 0.1	1.1 \pm 0.1
S (mg g $^{-1}$)	0.8 \pm 0.1	1.0 \pm 0.1	0.9 \pm 0.1
Na ($\mu\text{g g}^{-1}$)	236.0 \pm 5.0	253.7 \pm 20.2	366.3 \pm 88.5
Mn ($\mu\text{g g}^{-1}$)	411.0 \pm 33.1	418.7 \pm 64.8	336.3 \pm 67.9
C/N ratio	85.2 \pm 2.8^a	58.8 \pm 5.5^b	44.3 \pm 2.5^b
N/P ratio	23.6 \pm 2.8	26.3 \pm 2.6	39.1 \pm 5.7
Lignin/N ratio	45.6 \pm 2.1^a	32.9 \pm 1.7^b	31.2 \pm 1.6^b

Table 3

Characterization of the soil microcosms treatments (No litter, Control, 40N and 80N microcosms) at the beginning of the experiment; pH (H₂O); OM, organic matter; C, total carbon; total N, nitrogen; P, extractable phosphorus; K, extractable potassium; Mg, extractable magnesium; C/N ratio; N/P ratio; and C/P ratio. Different letters refer to statistically significant differences between treatments (ANOVA $p < 0.05$ followed by a Bonferroni test) and are shown in bold. Values represent the mean ($n = 3$ microcosms per treatment) \pm SE.

	No litter microcosm	Control microcosm	40N microcosm	80N microcosm
pH (H ₂ O)	5.5 \pm 0.0^a	5.3 \pm 0.0^b	5.1 \pm 0.0^c	5.2 \pm 0.0^{bc}
OM (%)	6.9 \pm 0.0^b	8.1 \pm 0.5^{ab}	8.2 \pm 0.0^{ab}	8.4 \pm 0.2^a
C (mg g ⁻¹)	20.4 \pm 1.5^b	25.6 \pm 1.4^a	25.6 \pm 1.6^a	25.4 \pm 1.6^a
N (mg g ⁻¹)	1.0 \pm 0.1	1.0 \pm 0.1	1.1 \pm 0.1	1.1 \pm 0.1
P (μ g g ⁻¹)	8.3 \pm 1.5	11.0 \pm 1.4	11.8 \pm 1.4	11.4 \pm 1.1
K (μ g g ⁻¹)	142.3 \pm 10.3	152.5 \pm 10.7	156.9 \pm 11.0	156.5 \pm 10.4
Mg (μ g g ⁻¹)	111.7 \pm 10.9	123.8 \pm 11.5	126.2 \pm 9.7	123.0 \pm 9.4
C/N ratio	21.0 \pm 0.6^b	25.0 \pm 0.7^a	24.3 \pm 0.7^a	23.6 \pm 0.5^{ab}
N/P ratio	120.9 \pm 12.7	94.8 \pm 6.7	90.7 \pm 6.4	95.3 \pm 3.8
C/P ratio	2537 \pm 247	2375 \pm 200	2200 \pm 127	2246 \pm 74

soil plus litter, were analysed for pH (H₂O), OM and concentrations of total C, N, extractable P, K and Mg as described by Dias et al. (2011a, 2012). At the end of the experiment, microcosms were analysed for pH (H₂O), total C and N concentration, nitrate (NO₃⁻–N – Matsumura and Witiaksono, 1999), ammonium (NH₄⁺–N – Cruz and Martins-Loução, 2000) and phosphate (PO₄³⁻–P – Fiske and Subbarow, 1925) as described in Dias et al. (2011a, 2012). Soil inorganic N (inorgN) was determined as the sum of the water extracted NH₄⁺ and NO₃⁻. NO₃⁻, NH₄⁺, inorgN and PO₄³⁻ were expressed as μ g N or P per gram of dry soil.

2.5. Soil microbial PLFA extraction and quantification

Soil was taken from the microcosms at the end of the incubation and kept at -20°C until freeze-dried at -20°C . Soil microbial composition was determined on 1 g of freeze-dried soil, using phospholipid fatty acids (PLFA) analysis by the Bligh and Dyer method (1959), adapted by White et al. (1979) and described in Treonis et al. (2004). The lipid extract was fractionated on silicic acid columns into different polarity classes by sequential elution with chloroform, acetone and methanol. A C19:0 internal standard was added before the methylation step and used for calculating retention times and quantification. Samples were analysed using a gas chromatogram (Agilent 5890GC) equipped with a flame ionisation detector and capillary column (Varian CP Sil 5 CB) using the following oven conditions: initially 50°C for 5 min, followed by a gradual increase of $10^{\circ}\text{C}/\text{min}$ up to 270°C , then $3^{\circ}\text{C}/\text{min}$ up to 320°C and held at 320°C for 10 min. Fatty acids were identified by retention time in comparison with previously identified samples and by GC–MS on an Agilent 6890GC connected to an Agilent 5973 Mass Selective Detector. Total PLFA concentration was calculated using all identified PLFA.

2.6. CO₂ and N₂O fluxes

At the end of the experiment (3 months), 100 g fwt samples of soil at 60% of water-filled pore space from each microcosm (three per treatment) were placed into sealed perspex columns (20 cm height \times 5 cm diameter) in a growth chamber, in the dark at 25°C , as described by Sánchez-Martín et al. (2008) in order to measure CO₂ and N₂O fluxes. CO₂ and N₂O concentrations were measured after 24 h of incubation, directly through a sampling port fitted into the air outlet tap, using a trace gas analyser (TGA – 1412 Photoacoustic Field Gas-Monitor; Innova AirTech Instruments, Ballerup, Denmark) as described by Fanguiero et al. (2008). The CO₂ and N₂O

fluxes were calculated as the differences between the concentrations in the airflow before and 30 min after closing the columns (Sánchez-Martín et al., 2008).

2.7. Calculations and statistical analysis

The variations in the concentration of soil OM content (ΔOM – Fig. 2) and of total C (ΔC – data not shown) and N (ΔN – data not shown) were calculated as the percentage of variation in the concentration of the given variable during the experiment, in relation to its initial concentration:

$$\Delta\text{parameter}(\%) = \frac{(\% \text{Parameter}_{\text{end}} - \% \text{Parameter}_{\text{beginning}})}{\% \text{Parameter}_{\text{beginning}}} \times 100$$

Summary statistics of litter, microcosms and gas parameters were compared for the different treatments. A two-way ANOVA was used to assess the existence of significant interactions between plant functional group (PFG) and N addition treatments for leaf-litter composition. Differences per treatment in litter and microcosms parameters were analysed by a one-way ANOVA, followed by a Bonferroni test ($p < 0.05$), or by a Games–Howell test whenever homogeneity of variances was not confirmed by the Levene's test. Linear correlations between the indicators of soil OM decomposition (ΔOM and ΔC ; and CO₂ and N₂O fluxes) and factors influencing soil OM decomposition were examined using Pearson's correlations. In all cases, analyses were performed using SPSS software, version 20.0.

3. Results

3.1. Ecological N-driven interactions between litter traits

Leaf-litter produced by the standing plant communities receiving the distinct N field treatments was mainly composed of leaves from summer semi-deciduous species (mostly *C. ladanifer* and *Cistus salvifolius*), with a smaller contribution from evergreen sclerophylls (Fig. 1; e.g. *M. communis*, *Q. coccifera*, *P. lentiscus*). The N field treatments altered the proportion of summer semi-deciduous ($F_{2,6} = 82.3$, $p = 0.000$), evergreen sclerophylls ($F_{2,6} = 47.3$, $p = 0.000$) and graminoids ($F_{2,6} = 108.0$, $p = 0.000$) in the leaf-litter. The leaf-litter produced under the higher N dose (80N litter) differed in relation to the remaining N field treatments in the proportion of the two plant functional groups (PFG; summer semi-deciduous and evergreen sclerophylls), and added a new one,

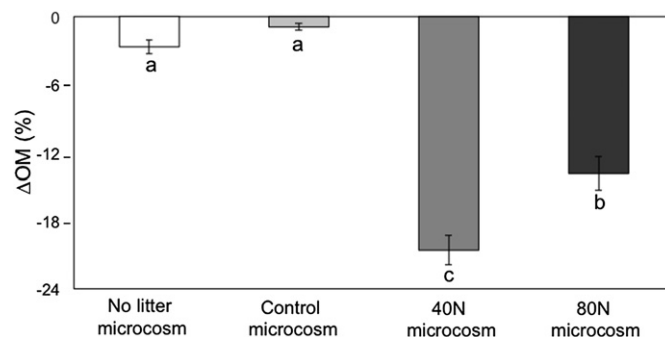


Fig. 2. Loss of OM between the beginning and the end of the experiment [ΔOM (%)] according to the microcosms treatments (No litter, Control, 40N and 80N microcosms). Different letters refer to significant differences between treatments (ANOVA $p < 0.05$ followed by a Bonferroni test). Bars represent the mean ($n = 3$ microcosms per treatment) \pm SE.

the graminoids. In comparison to the leaf-litter produced by the standing community from the control plots (no N addition), leaf-litter produced under the higher N dose treatment had 30% less litter from summer semi-deciduous species, while that of evergreen sclerophylls doubled and grasses (e.g. *Brachypodium phoenicoides*, *Gastridium ventricosum*) 'appeared' (~10%). The leaf-litter produced under the 40N field treatment was similar in composition and proportion of the PFG to that produced by the Control (Control litter).

Whole community leaf-litter was also assessed for N-driven changes in its chemical composition. Compared to the other treatments, the intensity of the *O*-alkyl-C region (45–110 ppm – polysaccharides) decreased in 80N litter ($F_{2,6} = 37.6$, $p = 0.000$, [Supplementary data Table 1](#)) indicating a reduction of these molecules. Since recalcitrant compounds of OM mainly control lignin rate decomposition, a low value of e.g. *O*-alkyl-C/Aromatic C means that when comparing with other sample at the same time in that particular sample the *O*-alkyl-C compounds were more degraded than the others (e.g. Aromatic C). In this case, in the 80N litter there was more degradation of *O*-alkyl-C compounds while lignin like compounds (e.g. aromatic C) were accumulated. And that is confirmed by the increase in lignin concentration ([Table 1](#)). Other important regions where intensity increased in the 80N litter were the aromatic ($F_{2,6} = 8.9$, $p = 0.016$), phenolic ($F_{2,6} = 27.8$, $p = 0.001$) and carboxyl ($F_{2,6} = 19.6$, $p = 0.002$). Changes in the litter lignin concentration ($F_{2,6} = 14.9$, $p = 0.005$), aromaticity ($F_{2,6} = 15.5$, $p = 0.004$) and the *O*-alkyl C to Aromatic C ratio ($F_{2,6} = 14.3$, $p = 0.005$), revealed differences in the quality of 80N litter compared with Control and 40N litters ([Table 1](#)). Given that the microcosms' C concentrations were below the detection limit of the ^{13}C CPMAS NMR procedure, it was not possible to monitor the changes that occurred in the lignin and aromaticity contents during the decomposition experiment.

Irrespective of the N additions, the litter produced by the standing maquis vegetation had low concentrations of N and P ([Table 2](#)). N field additions (40N and 80N) did affect some litter traits: N ($F_{2,6} = 18.1$, $p = 0.003$) and potassium ($F_{2,6} = 7.9$, $p = 0.021$) concentrations and the C/N ($F_{2,6} = 29.1$, $p = 0.001$) and lignin/N ($F_{2,6} = 19.5$, $p = 0.002$) ratios. 40N and 80N litters had higher N and K concentrations than the Control litter, and lower C/N and lignin/N ratios than the Control litter. The remaining analysed nutrients (as well as some of their ratios) were not significantly affected by the N treatments.

At the beginning of the decomposition experiment, the microcosms had similar total N concentrations ($F_{3,8} = 0.2$, $p = 0.915$) although their C/N ratios differed ($F_{3,8} = 8.3$, $p = 0.008$): the No litter microcosm had the lowest C/N ratio while Control and 40N microcosms had significantly higher C/N ratios and 80N microcosm did not significantly differ from the other treatments ([Table 3](#)).

3.2. Consequences of the altered litter traits on organic matter decomposition

Although the losses of OM (ΔOM) and of C (ΔC) followed a similar pattern, being strongly and positively correlated ($r = 0.80$, $p = 0.002$; [Table 4](#)), comparison of their correlations with litter and soil characteristics that typically influence decomposition ([Table 4](#)) showed that ΔOM was more related with litter and soil parameters than ΔC . Taking ΔOM from the microcosms as an indicator of decomposition, significant differences between the treatments ($F_{3,8} = 80.7$, $p = 0.000$) were observed: 40N microcosm lost ~20% of its initial OM, thus resulting in the highest decomposition rate ([Fig. 2](#)); 80N microcosm resulted in intermediate ΔOM (~12% of initial OM concentration); and No litter and Control microcosms showed the lowest ΔOM (<5% of initial OM concentration), i.e. the

Table 4

Pearson's correlations between the indicators of OM decomposition (ΔOM , ΔC and CO_2 and N_2O fluxes) and the potential factors influencing OM decomposition. *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

	ΔOM	ΔC	CO_2 flux	N_2O flux
ΔOM	1.00	0.80**	−0.58**	−0.83**
ΔC	0.80**	1.00	−0.22 ns	−0.46 ns
ΔN	0.03 ns	0.26 ns	0.26 ns	0.28 ns
Litter [N]	−0.62*	−0.57 ns	−0.30 ns	0.22 ns
Litter C/N ratio	0.71*	0.61 ns	0.20 ns	−0.32 ns
Litter [lignin]	−0.20 ns	−0.26 ns	−0.67 ns	−0.24 ns
Litter [lignin]/[N]	0.84**	0.75*	−0.00 ns	−0.48 ns
Soil C/N ratio	−0.26 ns	−0.04 ns	0.56 ns	0.42 ns
$\Delta [\text{H}^+]$	0.78**	0.38 ns	−0.73**	−0.80**
CO_2 flux	−0.58**	−0.22	1.00	0.87**
N_2O flux	−0.83**	−0.46 ns	0.87**	1.00
$[\text{NO}_3^-]$	0.37 ns	0.12 ns	−0.83**	−0.65**
$[\text{NH}_4^+]$	0.35 ns	0.01 ns	−0.68**	−0.63**
[inorgN]	0.38 ns	0.11 ns	−0.85**	−0.68**
$[\text{PO}_4^{3-}]$	−0.74**	−0.67*	0.04 ns	0.37 ns
Available N/P	0.74**	0.44 ns	−0.71**	−0.77**
Litter [P]	−0.47 ns	−0.19 ns	0.43 ns	0.63 ns
Litter [Mn]	0.05 ns	0.03 ns	0.30 ns	0.12 ns
Litter C/P ratio	0.47 ns	0.24 ns	−0.37 ns	−0.57 ns
Litter N/P ratio	−0.31 ns	−0.44 ns	−0.55 ns	−0.17 ns
Soil [C]	−0.36 ns	−0.03 ns	0.42 ns	0.44 ns
Soil [N]	−0.14 ns	0.04 ns	0.10 ns	0.12 ns
Soil [P]	−0.36 ns	−0.09 ns	0.39 ns	0.35 ns
Total PLFA	−0.22 ns	0.03 ns	0.83**	0.51 ns
Fungal PLFA	−0.31 ns	−0.06 ns	0.70*	0.49 ns
Bacterial PLFA	−0.13 ns	0.04 ns	0.67*	0.36 ns

lowest decomposition rates. Considering leaf-litter chemical characteristics, the highest correlation was between litter's lignin/N ratio and microcosms' ΔOM ($r = 0.84$, $p = 0.005$, [Table 4](#)). There was no significant ΔN during the experiment or between the treatments ($F_{3,8} = 3.3$, $p = 0.090$).

Addition of different litters significantly altered the soil microbial community structure (total PLFA, $F_{3,8} = 12.3$, $p = 0.004$; bacterial PLFA, $F_{3,8} = 4.2$, $p = 0.046$; fungal PLFA, $F_{3,8} = 6.5$, $p = 0.016$ – [Fig. 3](#) and [Supplementary data Table 2](#)) and activity (CO_2 fluxes, $F_{3,8} = 80.2$, $p = 0.000$; N_2O fluxes, $F_{3,8} = 31.7$, $p = 0.000$ – [Fig. 5](#)). In general, the analysed PLFA were more abundant in 40N microcosm, followed by Control and No litter microcosms and were depressed by the litter from the high N treatment (80N microcosm). The exceptions were C16:1 ω 7 (Gram negative bacteria, $F_{3,8} = 1.0$, $p = 0.433$), C17:1 ω 8 (bacteria, $F_{3,8} = 3.5$, $p = 0.068$) and C18:1 ω 7 (Gram negative bacteria, $F_{3,8} = 2.5$, $p = 0.133$) for which there were no significant differences ([Supplementary data Table 2](#)). Given that the majority of the analysed PLFA followed a similar pattern, it was not possible to identify a biomarker for changes in litter traits. Moreover, total microbial PLFA followed the same pattern as individual PLFA, with bacteria dominating the microcosms' microbial communities. The patterns of total bacterial PLFA ([Fig. 3b](#)) and of total fungal PLFA ([Fig. 3c](#)) were similar to that of total PLFA ([Fig. 3a](#)). The availability of N per unit of soil PLFA also distinguished between 80N microcosm and the other treatments ($F_{3,8} = 78.7$, $p = 0.000$ – [Fig. 4](#)). Microbial community structure (total PLFA and total bacterial and fungal PLFA) was strongly correlated ($r > 0.6$, $p < 0.05$) with CO_2 fluxes between microcosms' soil and atmosphere, but not with N_2O fluxes ([Table 4](#)).

The functional changes due to altered litter composition ([Fig. 1](#) and [Tables 1](#) and [2](#)) in soil microbial activity were assessed by measuring CO_2 and N_2O fluxes between soil and atmosphere ([Fig. 5](#)). Soil respiration was significantly higher in 40N microcosm while N_2O fluxes increased (less potential to act as N_2O sink). Fluxes

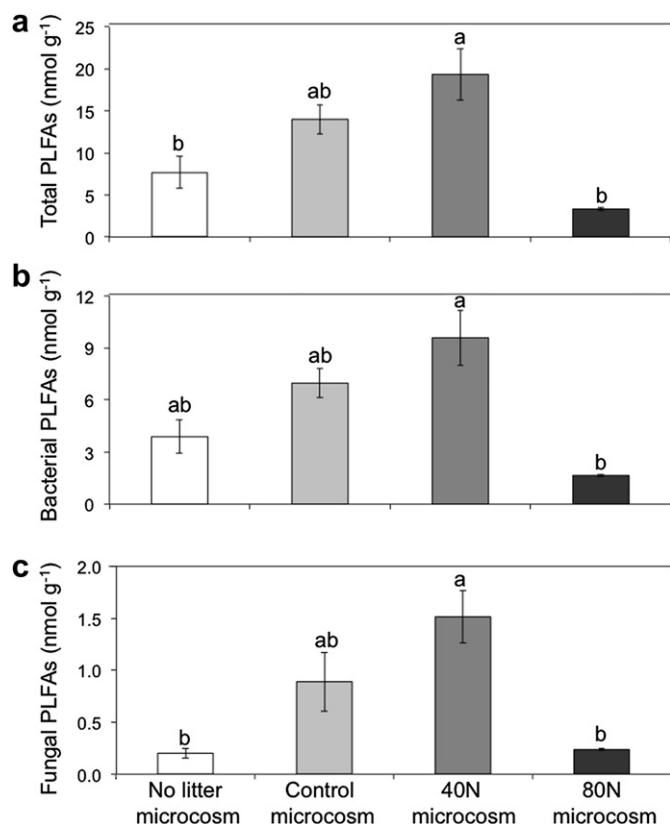


Fig. 3. Microcosms' total (a), bacterial (b) and fungal (c) PLFA concentrations determined at the end of the experiment and according to the treatments (No litter, Control, 40N and 80N microcosms). Different letters refer to statistically significant differences between treatments (ANOVA $p < 0.05$ followed by a Bonferroni test). Bars represent the mean ($n = 3$ microcosms) \pm SE.

of these two gases were strongly correlated ($r = 0.87$, $p = 0.000$). CO_2 and N_2O fluxes were strongly correlated with ΔOM , the availability of nitrate and inorganic N, available N/P, while CO_2 fluxes were strongly correlated with microbial community structure (PLFA – Table 4).

In general, OM decomposition (e.g. ΔOM) was not correlated with litter traits (e.g. litter C/N ratio) or initial microcosm characteristics (e.g. microcosm initial P concentration). However, there were significant correlations with the following litter traits: N concentration and lignin/N and C/N ratios (Table 4). For the CO_2 and N_2O fluxes, the strongest correlations were with the final

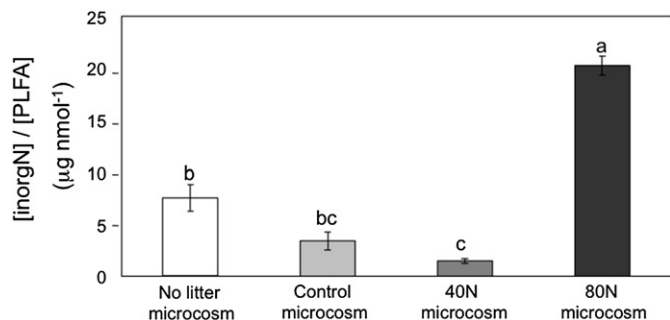


Fig. 4. Microcosms' availability of inorganic N per total microbial PLF at the end of the experiment and according to the treatments applied (No litter, Control, 40N and 80N microcosms). Different letters refer to statistically significant differences between treatments (ANOVA $p < 0.05$ followed by a Bonferroni test). Bars represent the mean ($n = 3$ microcosms) \pm SE.

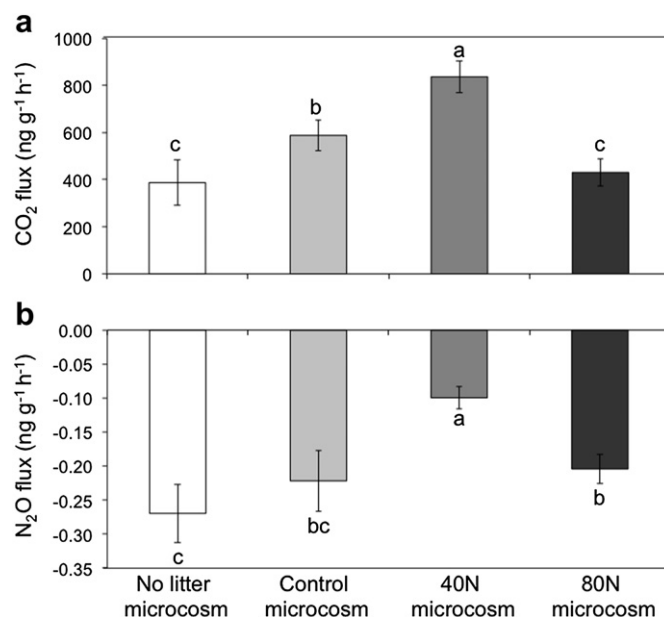


Fig. 5. CO_2 (a) and N_2O (b) net fluxes between soil and the atmosphere at the end of the experiment and according to the treatments applied (No litter, Control, 40N and 80N microcosms). Different letters refer to statistically significant differences between treatments ($p < 0.05$ followed by a Bonferroni test). Bars represent the mean ($n = 3$ microcosms) \pm SE.

concentrations of NO_3^- , NH_4^+ , total inorganic N and the ratio between inorganic N and phosphate (available N/P).

4. Discussion

4.1. Ecological N-driven interactions between litter traits

Increased N availability can change biodiversity in any type of ecosystem (Sala et al., 2000; Bobbink et al., 2010; Dias et al., 2011a, 2012), perhaps by altering the competitive interactions between species (Bobbink et al., 2010). In Mediterranean ecosystems, dominated by evergreen sclerophylls and summer semi-deciduous species, evergreen sclerophylls species are more conservative in respect to nutrients and more water use efficient (Correia and Catarino, 1994; Canadell et al., 1996; Dias et al., 2011b), and may therefore have advantage over the summer semi-deciduous under conditions (such as increased N availability) that decrease P and/or water availability to individual plants (Craine, 2009). Summer semi-deciduous and evergreen sclerophylls also differ in their strategy to use N; evergreen sclerophylls invest more N in the synthesis of structural compounds (e.g. lignin) which might increase stress tolerance (e.g. water stress, herbivory, etc – Craine, 2009). Due to the higher lignin concentration of evergreen sclerophyll leaves (in relation to summer semi-deciduous – Schlesinger and Mavis, 1981; Fioretto et al., 2005), the increased proportion of evergreen sclerophylls in the leaf-litter produced under the higher N dose (Fig. 1) is likely to have led to the higher lignin concentrations in the corresponding leaf-litter (80N litter – Table 1). As a result, when increased N availability changes the balance between plants, groups of plants or litter, that have distinct leaf traits, as occurred in the 80N treatment, effects on decomposition may be expected. However, N field additions also resulted in increased litter N concentration and lower C/N ratios (Table 2), which can facilitate litter decomposability (Vourlitis et al., 2009; Dias et al., 2012). Therefore, assessing the effects of increased N availability on decomposition must account for the interactions between higher lignin and higher N concentrations.

4.2. Consequences of altered litter traits on organic matter decomposition

OM decomposition (Δ OM), soil microbial biomass and activity (Figs. 2, 3 and 5) in the microcosms with leaf-litter produced under low N availability (Control microcosm) or without litter (No litter microcosm) did not differ. However, adding leaf-litter produced under increased N availability (40N and 80N microcosms) altered decomposition rates, confirming the importance of the interaction between plant community change and plant–soil interactions in nutrient-poor ecosystems (such as this Mediterranean maquis), and most importantly reinforces the role of N availability in controlling ecosystem nutrient cycles.

Soil C/N ratio is regarded as a determinant parameter in OM decomposition (Davidson and Janssens, 2006). As a general rule, net mineralization occurs at C/N ratio <20 (Craine, 2009). The fact that soil microcosms had C/N ratios in the range 21–25 (Table 3) may imply that within that range, changes in the C/N ratio are not likely to interfere with decomposition, as shown by the lack of correlation between soil C/N ratio and Δ OM (Table 4). In fact, the lower loss of OM observed in the 80N microcosm may reflect the higher aromaticity and lignin concentration of the litter mixture in comparison with the other N addition treatment (40N microcosm – Fig. 2 and Table 1). Lignin is known to be relatively resistant to microbial decomposition (Austin and Ballaré, 2010) and breaks down to produce aromatic phenolic compounds that suppress hydrolytic enzyme activity crucial to decomposition (Freeman et al., 2001). It is likely that the effect of this lignin-driven suppression has overcome the stimulatory effect of leaf-litter's increased N concentration and may explain why OM decomposition in 80N microcosm was lower than in 40N microcosm (Fig. 2). In agreement, litter's lignin/N ratio (Table 2), a commonly used indicator of litter decay dynamics (Aber and Melillo, 1982; Aber et al., 1990; Knorr et al., 2005), was the litter trait most related with microcosms' Δ OM (Table 4). This may be related with the importance of the N sources, namely of nitrate, in the regulation of secondary metabolism, such as the phenylpropanoid metabolism responsible for the synthesis of lignin (Fritz et al., 2006). It is therefore possible that the litter lignin/N ratio may not be an indicator of decomposability when changes in litter quality are driven only by ammonium deposition (e.g. close to agricultural sources). Our results show that at the community/ecosystem level there is an interaction between the positive effect of higher litter N concentration on decomposability and the negative effect of increased litter lignin content.

Soil N and P availability (Table 4 and Fig. 4) reduced soil microbial biomass (Fig. 3) and activity (Fig. 5) in the 80N microcosm treatment, reflecting the changes in leaf-litter composition (Fig. 1 and Tables 1 and 2). This is evidence of the influence of plant community richness and composition (including species and community litter traits) on soil microbial community structure and therefore on OM decomposition (e.g. De Deyn et al., 2008; Fornara et al., 2009).

The microbial activity indicated by the net CO₂ and N₂O fluxes from the microcosms was within the range reported from field-studies in the Mediterranean Basin (Rosenkranz et al., 2006). It has been shown that Mediterranean forest soils are mostly weak N₂O emitters (<10 $\mu\text{g N m}^{-2} \text{ h}^{-1}$) or even temporal sinks for atmospheric N₂O (Fenn et al., 1996; Bernal et al., 2003; Butterbach-Bahl and Kiese, 2005), which has been linked to their very low N availability (Rosenkranz et al., 2006). The pattern of net CO₂ and N₂O fluxes (very small in the 80N microcosm and highest for 40N microcosm – Fig. 5) appear to reflect differences in OM decomposition (Fig. 2 and Table 4) and the size of the soil microbial communities (Fig. 3 and Supplementary data Table 2). However, the

lack of correlation between total PLFA and N₂O fluxes (Table 4) reflects the fact that only a specific group of microorganisms (i.e., nitrifiers consume N₂O in nitrifier denitrification – Chapuis-Lardy et al., 2007) was responsible for the observed negative fluxes (Fig. 5).

5. Conclusions

Data show that N additions reduced the abundance of summer semi-deciduous species and benefited evergreen sclerophylls and graminoids. Which resulted in a change in the quality of litter inputs, reducing the size and activity of the soil microbial biomass, and suppressing soil OM decomposition rates, despite high N levels. Although it is important to also quantify *in situ* leaf-litter decomposition (e.g. using litter bags), which would also account for the N-driven changes in the soil microbial community, after 4.5 years of our N-manipulation field experiment, there were no significant differences in litterfall, though soil OM concentration was higher in the 80N plots (Dias et al., unpublished). Thus, the accumulation of OM in the field may support the slower decomposition rates observed in the laboratory. In addition, under natural conditions, OM accumulates much more in the soil patches under the influence of evergreen sclerophylls (~16%) than under that of summer semi-deciduous (~10% – Cruz et al., 2008). Therefore, taking into account the estimates of increased N deposition for semi-natural Mediterranean Basin ecosystems, it is possible that the ecological niche occupied by summer semi-deciduous species will narrow (Dias et al., 2011b) while that of evergreen sclerophylls widens, which may lead to lower litter decomposition rates (Fig. 3) and an overall accumulation of OM. These results confirm the importance of the interaction between plant community change and plant–soil interactions in nutrient-poor ecosystems, such as this Mediterranean maquis, and highlight the important role of N availability in controlling ecosystem nutrient cycles.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2012.10.027>.

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