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ABSTRACT

Soil C and N dynamics were evaluated in five eucalypt plantations within a precipitation gradient (500-2000 mm) in Portugal. Soil physical and chemical properties, total and labile (particulate organic matter, hydrolyzable, hot water soluble and microbial) soil C and N pools, and C and N mineralization were measured to characterize the C and N dynamics and their controlling factors within this gradient. Contents of total and labile soil organic C and N were positively correlated with the mean annual precipitation. A similar relationship was observed for net N mineralization (anaerobic and long-term aerobic incubation), gross N mineralization (¹⁵N isotope dilution technique) and C mineralization. In contrast, rates of C and N mineralization (per unit of C and N) were higher in the driest sites due to their higher proportion of particulate organic matter C. Net and gross N mineralization were strongly correlated and showed similar controlling factors (mean annual precipitation, total and labile C and N and extractable P contents), suggesting that net N mineralization during long-term aerobic incubation reflects gross N transformations. Although, gross NO₃-N production and gross NO₃-N immobilization were observed in all sites, net nitrification in the drier sites was not observed in the first weeks of the study. Our results suggest that, under Mediterranean conditions, mean annual precipitation is the major factor determining the C and N dynamics in soils with *Eucalyptus* plantations.

Keywords: N cycling; Mediterranean climate; ¹⁵N isotope pool dilution; labile soil organic matter; understory

Introduction

In the Iberian Peninsula, plantations of *Eucalyptus globulus* Labill. cover about 1.3x10⁶ ha, 54% of which are located in Portugal (DGCN 2000; IFN 2006). These plantations are intensively managed as short rotations coppiced stands and their sustaining productivity may be largely dependent on soil fertility enhancement by using proper management systems (Jones et al. 1999; Merino et al. 2003; Madeira et al. 2004). To evaluate the use of these systems, the patterns, controls, and variability of C and N cycling should be better understood (Mendham et al. 2002; O'Connell et al. 2004).

Carbon cycling is mostly regulated by water balance through the production and decomposition of plant litter (Austin and Sala, 2002). Likewise, soil N cycling is influenced by precipitation in terms of soil N losses

(Holtgrieve et al. 2006) and soil N transformations (Douglas et al. 1998; Meyer et al. 2006). In addition to climate, the amount of N mineralized depends on several soil factors such as N content, C/N ratio and labile fractions of organic matter (Stevenson 1986; Mendham et al. 2004). At regional scale, these factors widely vary affecting N mineralization even under similar vegetation cover (Zak and Pregitzer 1990). Also, the contribution of nitrification to the pool of available inorganic nitrogen is extremely variable, as well as the explanations for such variability (Attiwill and Adams 1993).

For Australian eucalypt forest soils, Attiwill et al. (1996) reported that N availability is dominated by the cycling of organic matter, and soil C/N ratio may provide an index of N net mineralization, which is insignificant above a ratio of 30. These authors also stated that

ammonification predominates in the C/N range 15-30, while nitrification occurs at ratios below 15. In fact, ammonification has been found to be largely predominant in eucalypt soils over a wide range of soil C/N ratio (12-22) and climate conditions, including temperate (Polglase et al. 1992) and tropical (Gonçalves et al. 1999; Bernhard-Reversat 1996) climates. Despite the importance of N availability on productivity of *Eucalyptus* plantations (Adams et al. 1989, Madeira and Pereira 1990-91), the causes of low nitrification in eucalypt soils are not well understood and other factors have been mentioned as possible controls such as low substrate C (Ellis and Pennington 1989) and $\text{NH}_4\text{-N}$ (Polglase et al. 1992) availability, allelopathic inhibition (Ellis and Penninton 1989), low populations of autotrophic nitrifiers (Bauhus et al. 1993), and soil pH (Ste-Marie and Paré 1999).

Nitrogen mineralization studies in forest soils have been usually based on net measurements since it is a useful indicator of N availability for plant uptake (Hart et al. 1994; Schimel and Bennett 2004). Net nitrification represents the balance between gross nitrification and gross $\text{NO}_3\text{-N}$ immobilization (Davidson et al. 1992), but it does not show the actual production of $\text{NO}_3\text{-N}$, and the lack of net nitrification may not coincide with low gross nitrification. Indeed, high gross nitrification and $\text{NO}_3\text{-N}$ immobilization have been reported in forest soils with low $\text{NO}_3\text{-N}$ concentrations and slow net nitrification (Davidson et al. 1992; Stark and Hart 1997). Therefore, N dynamics should be better understood if gross N transformations were measured.

In the Iberian Peninsula, eucalypt plantations exhibit variable productivity and occur in a wide range of precipitation and understory plant species composition. Soils under these plantations have a wide range of organic matter contents (Magalhães 2000; Merino et al. 2003) and variable (15-31) C/N ratio (Fabião et al. 1987; Madeira et al. 1989; Magalhães 2000; Merino et al. 2003), values being lower (about 15) in wetter areas for several parent materials (Merino et al. 2003). Although the information on their C and N dynamics is still scarce, it is known that in areas with low mean annual precipitation (about 600 mm) net N mineralization during short term aerobic incubations is strongly dominated by

ammonification (Madeira et al. 2004).

In this context, we hypothesized that patterns of C and N mineralization as well as nitrate contribution to N availability could show strong variation according to the climate prevailing in Mediterranean eucalypt plantations. For verifying this hypothesis, soil C and N pools (total and labile) and C and N mineralization were evaluated in five plantations selected within an annual precipitation gradient to (i) describe the patterns of C and N dynamics, (ii) compare the patterns of net and gross N mineralization, and (iii) identify possible factors controlling C and N dynamics.

MATERIALS AND METHODS

Site description

The study was carried out in five sites representing the broad range of mean annual precipitation (500 to 2000 mm) for *E. globulus* plantations growing in Portugal (Table 1). The climate is of Mediterranean type with cool, moist winters and hot, dry summers. Two sites were selected in areas with mean annual precipitation higher than 1000 mm, characterized by rolling topography. Three other plantations were selected in areas with precipitation below 1000 mm and landscape flat to gently undulating. For site details see Table 1. In the selected sites soils are mostly Cambisols (*sensu* WRB 2006) developed over schists, sandstones or granite. For details on soil characteristics see Table 2.

All sites were 10-12 year-old second rotation plantations previously managed as follows: (i) soil was ripped and harrowed for site preparation; (ii) seedlings were planted at a spacing of 3 m by 3 m and each one received 200 g of NPK (14-21-21) fertilizer (i.e. 31.0 kg ha⁻¹ of N, 21.0 kg ha⁻¹ of P and 41.0 kg ha⁻¹ of K) at planting; (iii) trees were also fertilized 4-5 years after planting, and 200 kg/ha of Nitrolusal (26% N) were applied; (iv) weed control was done by harrowing; and (v) after the first rotation clear cutting, harvest residues were kept on the soil surface and no additional fertilizer was applied. Sites showed a wide range of productivity, which was not correlated with mean annual precipitation (Table 1).

^aTable 1 Selected site characteristics of studied plantations. W-CS: Cabeço Santo; W-VT: Vale Travanca, D-PG: Penegal; D-PR: Pedra Rato; D-FR: Furadouro; MAP: mean annual precipitation; MAT: mean annual temperature; AL: altitude; PD: productivity

Site	Geographic location	Soil Type	Bedrock	MAP (mm)	MAT ¹ (°C)	AL (m)	Slope (%)	PD (m ³ ha ⁻¹ y ⁻¹)	Understory Species ²	Understory cover ³
W-CS	40° 30' N 8° 18' W	Humic Cambisol	Schists	1800	13.7 (7-21)	581	> 30	12.2	<i>Pterospartum tridentatum</i> L. (23.2), <i>Erica umbellata</i> L. (13.4), <i>Calluna vulgaris</i> L. (9.6), <i>Funaria hygrometrica</i> Hedw. (5.7), <i>Agrostis curtisii</i> Kerguelen (5.5)	59.4
W-VT	40° 13' N 8° 00' W	Humic Cambisol	Schists	1100	11.2 (8-20)	332	15-30	11.3	<i>Erica umbellata</i> L. (44.8), <i>Funaria hygrometrica</i> Hedw. (14.4), <i>Calluna vulgaris</i> L. (11.4), <i>Briza maxima</i> L. (2.3), <i>Cistus ladanifer</i> L. (1.8)	75.6
D-PG	39° 21' N 8° 53' W	Eutric Cambisol	Sandstone	900	15.5 (10-19)	135	0-15	17.1	<i>Briza maxima</i> L. (9.1), <i>Erica umbellata</i> L. (7.3), <i>Simethis mattiazzii</i> (Vandelli) Sacc. (4.6), <i>Asphodelus ramosus</i> L. (3.9), <i>Danthonia decumbens</i> L. DC (2.5)	38.1
D-PR	39° 19' N 7° 41' W	Dystic Cambisol	Granite	750	15.0 (9-24)	304	0-15	8.3	<i>Briza maxima</i> L. (11.6), <i>Cytisus grandiflorus</i> DC. (3.8), <i>Quercus coccifera</i> L. (3.5), <i>Ulex minor</i> Roth (1.4), <i>Piptatum miliaceum</i> L. Coss (1.0)	22.0
D-FR	39° 20' N 9° 13' W	Dystic Cambisol	Sandstone	600	15.2 (11-19)	30	0-15	19.0	<i>Pteridium aquilinum</i> L. Kuhn (22.5), <i>Rubus ulmifolius</i> Schott (7.7), <i>Brachypodium sylvaticum</i> Huds. Beauv (3.6), <i>Genista triachanthos</i> Brot. (2.9), <i>Cistus salviifolius</i> L. (2.6)	51.6

¹Minimum and maximum mean monthly temperature between brackets. ²Proportion of soil covered by the five more abundant species between brackets. ³Average proportion of soil covers by understory vegetation. The floristic survey was carried out in spring using the line interception method (Kent and Coker 1992). A 15 m tape was used six times in each plot.

Table 2 Main characteristics of the studied soils

Site	Sand	Silt	Clay	Org C g kg ⁻¹	Total N	C/N	NH ₄ -N mg kg ⁻¹	NO ₃ -N mg kg ⁻¹	pH H ₂ O	Ext.P mg kg ⁻¹	Ext.Al ³⁺ cmol _c kg ⁻¹	ECEC
W-CS	653 ±5 ^c	212 ±8 ^b	135 ±4 ^a	64.4 ±1.3 ^a	3.55 ±0.08 ^a	18.2 ±0.7 ^b	1.14 ±0.60 ^a	1.41 ±0.32 ^a	4.73 ±0.07 ^d	6.6 ±2.1 ^a	3.91 ±0.06 ^a	4.27 ±0.05 ^a
W-VT	629 ±6 ^d	242 ±5 ^a	129 ±3 ^a	27.5 ±1.0 ^b	2.09 ±0.07 ^b	13.2 ±0.9 ^c	0.60 ±0.25 ^{ab}	1.06 ±0.93 ^a	4.89 ±0.14 ^c	2.8 ±0.1 ^{ab}	2.21 ±0.04 ^b	2.86 ±0.07 ^{ab}
D-PG	771 ±6 ^a	120 ±2 ^d	109 ±4 ^b	9.8 ±0.7 ^d	0.50 ±0.06 ^c	19.8 ±2.2 ^b	0.48 ±0.10 ^b	0.35 ±0.13 ^b	5.70 ±0.14 ^a	1.2 ±0.1 ^b	0.05 ±0.01 ^e	2.25 ±1.03 ^b
D-PR	717 ±7 ^b	155 ±4 ^c	128 ±5 ^a	10.7 ±0.7 ^d	0.54 ±0.05 ^c	19.9 ±1.6 ^b	0.56 ±0.13 ^{ab}	0.23 ±0.05 ^b	5.09 ±0.10 ^b	4.3 ±0.1 ^{ab}	1.82 ±0.01 ^c	2.84 ±0.00 ^{ab}
D-FR	783 ±13 ^a	102 ±2 ^e	115 ±5 ^b	13.9 ±0.2 ^c	0.57 ±0.02 ^c	24.6 ±0.8 ^a	0.89 ±0.50 ^{ab}	0.16 ±0.15 ^b	5.05 ±0.10 ^b	3.5 ±0.2 ^{ab}	0.64 ±0.03 ^d	2.81 ±0.04 ^{ab}

ECEC: effective cationic exchange capacity

Values are means (n=4) ±S.D. Mean values in a column followed by the same letter are not significantly different as estimated by the Tukey test (P<0.05).

Sample collection

Sampling (in January) was carried out in permanent monitoring plots (30 m x 30 m) for inventory purposes. Plots were divided into 100 grid cells considering eucalypt trees as intersections points. At each plot, after removal of the forest floor litter layer, a soil core (0-10 cm depth, 65 mm diameter) was collected in the centre of 16 randomly selected cells; four samples were combined randomly to give a composite sample, resulting in four samples per plot. The field-moist soil was sieved (< 4.75 mm) and stored at 4 °C for less than three days before analyses. Sub-samples were air-dried and sieved (< 2 mm) for physical and chemical analyses, and for labile soil organic matter (SOM) fractionations.

Laboratory procedures

Physical and chemical properties

Soil pH was determined potentiometrically in distilled water (soil:solution ratio 1:2.5). Organic C was determined by wet oxidation and total N using Kjeldhal digestion (Digestion System 40, Kjeltec Auto 1030 Analyzer). Extractable P and K were obtained using the Egnér-Riehm method (Egnér et al. 1960). Exchangeable Ca, Mg, Na and K (extracted with 1 M NH₄OAC, adjusted at pH 7.0) and extractable Al (extracted with 1 M KCl) were measured by atomic absorption spectroscopy (Aanalyst 300, Perkin Elmer), while P was measured by colorimetry.

Fractionation of soil organic C and N

As the labile pool does not correspond to any single soil organic matter fraction that can be readily determined (Khanna et al. 2001), labile and recalcitrant pools of soil organic C and N were separated by physical (particulate organic matter), chemical (acid and hot water extraction) and biological (microbial biomass) techniques (Khanna et al. 2001) to provide complementary information.

After dispersing air-dried soil by shaking with distilled water (10 g / 100 mL), soil was sieved (< 50 mm) and particulate organic C (C_{POM}) and N (N_{POM}) were determined in the coarse fraction by wet oxidation and Kjeldhal digestion, respectively.

Air-dried soils (10 g) free of plant debris (removed by flotation with NaCl) were refluxed for 16 h with 50 mL of 6 M HCl (Sollins et al.

1999) and hydrolyzable C (Chyd) and N (Nhyd) were measured in the centrifuged hydrolysates with an autoanalyzer (Skalar FORMAC Combustion TOC/N), using a near infrared spectroscope and a chemiluminescence detector, respectively.

Hot water soluble C (Chw) and N (Nhw) were extracted with distilled water (10 g air-dried soil : 50 mL) at 80 °C for 60 min (Khanna et al. 2001) and, after centrifugation, organic C and total N in extracts were determined as above. Microbial C and N (C_{mic} and N_{mic}) were measured by the chloroform fumigation-extraction method (Vance et al. 1987). Organic C and total N were determined by autoanalyzer, as previously described. C_{mic} and N_{mic} were calculated as the difference between fumigated and non fumigated samples, using a K_{EC} and K_{EN} factor of 2.64 and 1.85, respectively (Brookes et al. 1985).

C and N mineralization

Carbon mineralization was measured during 32 weeks by incubating 50 g of soil at 25 °C in a 2 L sealed glass jar with a 30 mL trap of 0.5 M NaOH placed inside. Periodically, NaOH was sampled and titrated with 0.5 M HCl to phenolphthalein endpoint, after addition of 1.5 M BaCl₂. Metabolic quotient (qCO_2) was calculated as the ratio of hourly basal respiration after two days to the corresponding initial C_{mic} [$mg\ CO_2-C\ (g\ C_{mic})^{-1}\ h^{-1}$].

A long-term aerobic incubation was carried out to describe the dynamic of NO₃-N and NH₄-N production. As previous studies indicated that nitrification was negligible in some Portuguese eucalypt soils (Madeira et al., 2004), net N mineralization was also evaluated by anaerobic incubation.

Net N mineralization under aerobic conditions was evaluated periodically during a 32 weeks incubation. Approximately 1 kg (fresh weigh, <4.75 mm) of each composite soil sample was incubated in polyethylene bags, at 25 °C, 60% of water holding capacity in the dark. NO₃-N and NH₄-N before and after incubation periods were extracted by shaking 10 g of soil for 1 h with 2 M KCl (1:5 soil:solution ratio). Extracts were filtered and NO₃-N and NH₄-N were determined by a segmented flow autoanalyzer (Skalar, SAN^{plus} System, Breda), using the hydrazinium reduction and the modified Berthelot method, respectively (Houba

et al. 1994). Net N mineralization was calculated by the difference between NO₃-N and NH₄-N at each incubation time and their respective initial levels.

Net N mineralization under anaerobic conditions was measured in sub-samples of 5 g soil added with 12.5 mL of distilled water after 14 days of incubation at 40 °C in 50 mL bottles (Keeney 1982). After incubation, 12.5 mL of 4 M KCl was added to samples, shaken for 1h and filtered, and NH₄-N concentrations determined as above. Mineralizable N was calculated by the difference between the amount of NH₄-N before and after incubation.

Gross N mineralization was determined by the ¹⁵N isotope dilution technique (Hart et al. 1994). Six 25 g subsamples of each composite soil sample were amended either with 2.8 µg N kg⁻¹ of (¹⁵NH₄)₂SO₄ (98 atom % ¹⁵N in excess), for gross mineralization measurement, or with 0.8 µg N kg⁻¹ of ¹⁵NO₃K (98 atom % ¹⁵N in excess), for gross nitrification measurement. Within 15 min after ¹⁵N addition, half of the soil samples were extracted with 125 mL of 2 M KCl and filtered. The other set was incubated for 24 h (darkness; 25 °C) and extracted as above.

Extracts were analyzed using a diffusion method, at room temperature (Couto-Vázquez and González-Prieto 2006). The resulting (NH₄)₂SO₄ solutions were then acidified with 1 mL 0.005 M H₂SO₄ and dried at 60 °C in a vacuum oven (Mettler VO400, PM400). To accelerate the drying process, the oven was alternatively under vacuum (100 mb) and atmospheric pressure, the incoming air being bubbled into H₂SO₄ to trap possible traces of atmospheric NH₃. The (NH₄)₂SO₄ salts were then introduced into tin capsules and analyzed for ¹⁵N in an elemental analyser (Carlo Erba CNS 1508) coupled on line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C). Gross mineralization, nitrification and immobilization were calculated using the equations developed by Kirkham and Bartholomew (1954).

In order to compare soils with different C and N contents, rates of mineralized C and N were expressed per unit of initial organic C ($mg\ C-CO_2\ g^{-1}\ C$) and total N ($mg\ N\ g^{-1}\ N$).

Statistical analysis

Data were statistically analysed by one-way ANOVA and differences among means were determined at $P < 0.05$ using the Tukey multiple range test. Relationships between N mineralization and mean annual precipitation and soil properties were explored by simple linear regression ($n=5$). Statistical procedures were performed using Statgraphics plus 5.1 (Manugistics Inc.).

RESULTS

Soil physical and chemical properties

Soil organic C and total N contents were significantly higher in the wetter sites (W-CS and W-VT) than in the other sites (Table 2). The

highest C/N ratio was observed in the D-FR site and the lowest in the W-VT site. Soils were more acidic in wet sites. Extractable P was always low ($1.2 - 6.6 \text{ mg kg}^{-1}$). At sampling time (early winter) the majority of mineral N in drier sites (58% in D-PG, 71% in D-PR and 85% in D-FR) was present as $\text{NH}_4\text{-N}$, while $\text{NO}_3\text{-N}$ predominated in wetter sites (W-CS and W-VT).

Labile soil organic C and N pool

Particulate organic matter (50-2000 μm , POM) accounted for the largest amount of soil organic C (28% - 48%), while the most important N fraction was that of acid hydrolyzable N (45% - 69%, Table 3). The percentages of organic C and total N in the hot water soluble (2.2-4.6%) and microbial (1-2%) fractions were low.

Table 3 Pre- and post-incubation (32 weeks) C and N contents (g kg^{-1} soil) in particulate organic matter (C_{POM} and N_{POM}), acid hydrolysates (Chyd and Nhyd), hot water extracts (Chw and Nhw) and microbial biomass (Cmic and Nmic) and their percentage (between brackets) in relation to organic C and total N.

Site	C_{POM}	Chyd	Chw	Cmic	N_{POM}	Nhyd	Nhw	Nmic
Pre-incubation								
W-CS	20.2±3.0 ^a (31.3)	12.0±1.6 ^a (18.7)	1.44±0.33 ^a (2.2)	0.71±0.09 ^a (1.1)	1.75±0.14 ^a (49.3)	1.58±0.24 ^a (44.6)	0.11±0.02 ^a (3.0)	0.036±0.004 ^a (1.0)
W-VT	8.8±0.7 ^b (32.8)	7.1±0.9 ^b (25.7)	1.24±0.25 ^a (4.5)	0.33±0.06 ^b (1.2)	0.91±0.05 ^b (43.5)	1.21±0.15 ^b (57.7)	0.06±0.00 ^b (3.4)	0.014±0.004 ^b (0.7)
D-PG	3.6±0.3 ^c (38.5)	2.4±0.4 ^c (24.8)	0.30±0.02 ^b (3.1)	0.11±0.06 ^c (1.2)	0.15±0.01 ^c (29.6)	0.34±0.01 ^c (69.5)	0.02±0.00 ^c (4.6)	0.010±0.002 ^b (1.9)
D-PR	4.3±0.2 ^c (40.2)	2.2±0.3 ^c (20.9)	0.47±0.05 ^b (4.4)	0.22±0.03 ^{bc} (2.1)	0.20±0.04 ^c (37.2)	0.31±0.03 ^c (58.6)	0.02±0.00 ^c (3.3)	0.011±0.003 ^b (2.1)
D-FR	5.7±0.8 ^c (40.1)	2.9±0.4 ^c (21.1)	0.54±0.10 ^b (3.9)	0.22±0.09 ^{bc} (1.6)	0.17±0.05 ^c (30.5)	0.29±0.03 ^c (51.8)	0.02±0.00 ^c (4.0)	0.008±0.006 ^b (1.9)
Post-incubation								
W-CS	3.3±0.8 ^b (6.2)	13.3±1.1 ^a (25.2)	1.92±1.07 ^a (3.5)	0.52±0.06 ^a (1.0)	1.61±0.19 ^a (54.0)	1.43±0.25 ^a (46.8)	0.50±0.28 ^a (16.9)	0.018±0.004 ^a (0.6)
W-VT	1.5±0.5 ^c (6.9)	7.1±1.0 ^b (31.3)	1.72±0.27 ^b (7.6)	0.20±0.01 ^b (0.1)	0.71±0.06 ^b (42.6)	0.70±0.10 ^b (42.0)	0.36±0.04 ^a (21.8)	0.008±0.004 ^b (0.5)
D-PG	2.6±0.3 ^{bc} (45.5)	2.3±0.3 ^c (41.5)	0.18±0.03 ^c (3.2)	0.08±0.06 ^b (1.5)	0.10±0.01 ^c (37.0)	0.17±0.02 ^c (61.6)	0.03±0.01 ^b (7.5)	0.006±0.002 ^b (2.1)
D-PR	2.8±0.3 ^b (49.2)	2.7±0.3 ^c (47.4)	0.25±0.02 ^c (4.4)	0.16±0.09 ^b (2.7)	0.13±0.02 ^c (43.2)	0.15±0.02 ^c (50.4)	0.04±0.00 ^b (14.5)	0.005±0.002 ^b (1.5)
D-FR	5.7±0.4 ^a (45.3)	3.6±0.5 ^c (29.0)	0.41±0.01 ^c (3.3)	0.15±0.09 ^b (1.2)	0.13±0.01 ^c (23.8)	0.16±0.02 ^c (30.5)	0.08±0.01 ^b (14.8)	0.005±0.002 ^b (0.9)

Values are means ($n=4$) ±S.D. For each sampling, mean values in a column followed by the same letter are not significantly different as estimated by the Tukey test ($P < 0.05$).

Soils from wetter sites (W-CS and W-VT) had significantly higher pre-incubation contents of POM, hydrolyzable and hot water extractable C and N than those taken in drier sites (D-PG, D-PR and D-FR, Table 3). The highest microbial C and N contents were also observed in the wettest site (W-CS). However, the relative amounts of labile organic C and N did not exhibit a clear trend within the precipitation transect (Table 3).

Strong correlations were observed among Corg and C labile pools ($r=1.00$, $r=0.98$, $r=0.89$ and $r=0.97$, for C_{POM} , $Chyd$, Chw and $Cmic$, respectively) and among total N and N labile pools ($r=1.00$, $r=0.98$, $r=1.00$ and $r=0.93$, for N_{POM} , $Nhyd$, Nhw and $Nmic$, respectively).

C mineralization

Reflecting the behaviour during the whole incubation period, at the end of the incubation, cumulative C mineralization was statistically higher in the W-CS site ($3490 \text{ mg C-CO}_2 \text{ kg}^{-1} \text{ soil}$) than in the D-PG ($1352 \text{ mg C-CO}_2 \text{ kg}^{-1} \text{ soil}$) and D-PR ($1287 \text{ mg C-CO}_2 \text{ kg}^{-1} \text{ soil}$) sites (Fig. 1). However, C mineralization rates (cumulative C mineralized expressed per unit of initial total C) were significantly lower in wetter (W-CS and W-VT) than in drier (D-PG, D-PR and D-FR) sites (Table 4). Initial metabolic quotient ($q\text{CO}_2$) values were significantly higher in the D-PG than in the other sites (Table 4).

Fig. 1 Cumulative C mineralized ($\text{mg C-CO}_2 \text{ kg}^{-1} \text{ soil}$) during 32 weeks of incubation. Different letters indicate significant differences among means ($n=4$) at the $P=0.05$ level

Net N mineralization

Nitrogen mineralized under anaerobic conditions was significantly higher in wetter sites (W-CS and W-VT) than in the others (Table 5). However, rates of mineralized N (per unit of initial total N) were significantly greater (about 2.5 times) in drier sites. A positive correlation was observed between N mineralization and total and labile pools of C and N ($r>0.89$, $P<0.05$, Table 6).

Net ammonification was the dominant N process during the first weeks of aerobic

incubation, especially in sites with low precipitation (Fig. 2). Except in the W-CS site, a lag of 8-10 (D-PR and D-FR) or, even, 24 weeks (D-PG) was observed for net nitrification, which became the dominant process in all soils during the second half of the incubation. At the end of incubation, the cumulative net nitrification decreased significantly in the order $W\text{-CS} > W\text{-VT} \gg D\text{-FR} > D\text{-PR} \gg D\text{-PG}$. After 32 weeks, the cumulative net mineralization ranged from 5.0 to $150.6 \text{ mg N kg}^{-1} \text{ soil}$, and significantly higher values were also observed in the W-CS site (Table 5). Net mineralization rates (N

mineralized per unit of N) were significantly higher in the D-FR site than in the other sites. Net N mineralization and nitrification under

aerobic incubation were positively correlated with total C and N and most of the labile pools of C and N (except Chw and Nhyd, Table 6).

Table 4 Carbon mineralization rate (mg C-CO₂ g⁻¹ of initial C), initial metabolic quotient [*q*CO₂, mg C-CO₂ (g Cmic)⁻¹ h⁻¹] and rates of gross N mineralization and gross nitrification (mg N g⁻¹N day⁻¹). Different letters indicate significant differences among means at the *P*=0.05 level

Site	C mineralization rate	<i>q</i> CO ₂	Gross N mineralization rate	Gross nitrification rate
W-CS	55±18 ^b	2.3±0.8 ^b	1.72±0.36 ^c	0.01±0.03 ^b
W-VT	71±7 ^b	2.9±0.3 ^b	0.01±0.00 ^d	0.01±0.01 ^b
D-PG	145±19 ^a	13.9±2.2 ^a	1.70±0.64 ^c	0.01±0.01 ^b
D-PR	127±39 ^a	3.4±1.0 ^b	5.53±0.84 ^a	0.59±0.27 ^a
D-FR	170±22 ^a	5.0±1.3 ^b	3.26±0.92 ^b	0.11±0.04 ^b

Gross N mineralization

Gross N mineralization was the highest in the W-CS site, intermediate in the W-VT, D-PR and D-FR sites, and the lowest in the D-PG site (Fig. 3). Gross mineralization rates (N mineralized per unit of N) were significantly higher in the driest (D-PG and D-FR, Table 4) than in the other sites. Gross NH₄-N immobilization accounted for more than 40% of the mineralized N in the D-PR and D-FR sites, but was considerably less in the W-CS site (21%) and negligible in the W-VT and D-PG sites.

The highest gross nitrification was observed in the W-VT site (Fig 3). Gross NO₃-N immobilization exceeded gross nitrification in all cases, but especially in the W-CS and W-VT sites, the latter showing the highest gross NO₃-N immobilization. Although gross nitrification was not detected in the D-PG site after 24 h of incubation, this soil showed large NO₃-N immobilization.

As observed for net N mineralization determined by aerobic incubation, gross mineralization was positively correlated with total C and N (*r*=0.94 and *r*=0.90, respectively, Table 6) and most labile C and N pools (*r*>0.90, *P* <0.05). In contrast, no significant correlation was observed for gross nitrification and gross immobilization of NH₄-N or NO₃-N (data not shown).

DISCUSSION

Soil C dynamics

Soil organic C content in studied sites was positively correlated with the mean annual precipitation (*r*=0.96, *n*=5, *P*<0.01), as reported for several soil types in the Iberian Peninsula (Martins et al. 1995; Hontoria et al. 1999). A positive correlation was also obtained between mean annual precipitation and most pre-incubation C labile pools (C_{POM} *r*=0.94, Chyd *r*=0.95, Cmic *r*=0.89; *p*<0.05), as observed for Cmic contents within wide climate ranges in North America (Insam 1990) and in the Mongolian steppe (Li and Chen 2004). Although lower ratios of Cmic/SOC in wet than in dry areas were observed in different climate regions (Insam 1990; Sparling 1992; Franzluebbers et al. 2001; Li and Chen 2004), such a relationship was not observed in the present study, and the same was true for the relative amounts of Chw and Chyd. However, the proportion of C_{POM} was negatively correlated with the mean annual precipitation (*r*=-0.95, *p*<0.01). This latter result could be a consequence of a longer growing season and subsequent development of biologically active soil fractions in dry sites, such as suggested by Franzluebbers et al. (2001) for active SOM fractions (Cmic) under contrasting climatic conditions in North America.

Table 5 Cumulative net N mineralization (mg N kg^{-1} soil), net mineralization rates (mg N g^{-1} total N) and net nitrification rates ($\text{mg NO}_3\text{-N g}^{-1}$ total N) after 2 weeks of anaerobic incubation and 10 and 32 weeks of aerobic incubation. Values are mean $\pm$ S.D. ($n=4$). Different letters indicate significant differences among means ($n=4$) at the $P=0.05$ level

	Anaerobic incubation		Aerobic incubation 10-w				Aerobic incubation 32-w			
	mg N kg^{-1} soil	mg N g^{-1} total N	mg N kg^{-1} soil	mg N g^{-1} total N	$\text{mg NO}_3\text{-N g}^{-1}$ total N	mg N kg^{-1} soil	mg N g^{-1} total N	$\text{mg NO}_3\text{-N g}^{-1}$ total N	mg N g^{-1} total N	$\text{mg NO}_3\text{-N g}^{-1}$ total N
W-CS	24.2 $\pm$ 0.6 ^a	6.8 $\pm$ 0.2 ^b	62.0 $\pm$ 20.1 ^a	17.4 $\pm$ 5.6 ^b	15.2 $\pm$ 4.9 ^a	150.6 $\pm$ 25.4 ^a	42.2 $\pm$ 7.1 ^b	36.4 $\pm$ 4.6 ^{5b}		
W-VT	19.8 $\pm$ 0.4 ^b	9.5 $\pm$ 0.2 ^b	21.7 $\pm$ 13.0 ^b	10.4 $\pm$ 6.2 ^b	6.6 $\pm$ 8.5 ^b	44.9 $\pm$ 7.3 ^b	21.6 $\pm$ 3.5 ^c	21.5 $\pm$ 3.2 ^{2c}		
D-PG	13.0 $\pm$ 0.5 ^c	26.6 $\pm$ 1.6 ^a	6.7 $\pm$ 2.8 ^b	14.8 $\pm$ 6.2 ^b	-1.4 $\pm$ 1.3 ^b	5.0 $\pm$ 2.9 ^c	11.2 $\pm$ 6.4 ^c	12.2 $\pm$ 6.1 ^{1c}		
D-PR	12.7 $\pm$ 1.6 ^c	25.4 $\pm$ 3.2 ^a	9.5 $\pm$ 1.4 ^b	19.0 $\pm$ 2.8 ^b	2.4 $\pm$ 3.8 ^b	22.3 $\pm$ 6.5 ^{bc}	44.6 $\pm$ 12.9 ^b	41.0 $\pm$ 6.80 ^b		
D-FR	14.6 $\pm$ 0.9 ^c	28.8 $\pm$ 1.2 ^a	23.6 $\pm$ 1.8 ^b	42.9 $\pm$ 3.2 ^a	18.3 $\pm$ 4.5 ^b	39.6 $\pm$ 5.7 ^b	72.1 $\pm$ 10.4 ^a	71.8 $\pm$ 9.75 ^a		

Variation in C mineralization in the studied sites could be explained by the total and labile fractions of C, following the findings of Ahn et al (2009) for southeastern U.S. coastal plain sandy soils. The strong reduction observed in the post-incubation C_{POM} contents from wetter sites (see Table 3) suggest that microbial activity during decomposition was mostly localized in the particulate SOM, possibly due to greater substrate accessibility of this coarse fraction as reported by Parfitt and Salt (2001). Also, differences in the rates of C mineralized among sites may be mostly related to the relative amounts of C_{POM} ($r=0.89$, $p<0.05$), but not with those of other labile pools. Our results are in agreement with the positive relationship between C mineralization rates and C_{POM} contents reported for eucalypt soils (Mendham et al. 2004) and also for Mediterranean mountain soils (Oyonarte et al. 2008). Variability in C mineralization rate may not only be explained by different C availability (relative amounts of C_{POM}), but also by soil pH. In fact, the negative correlation of the C mineralization rate with extractable Al content ($r=0.91$; $p<0.05$) suggests that the higher C mineralization rate in the drier sites may be also due to their lower soil acidity.

Soil C storage in forest soils is determined by the balance between inputs from net primary productivity and outputs from decomposition (Kane et al. 2005). Although in similar environmental conditions SOC storage has been related to eucalypts productivity (Madeira et al. 2002), in our study it seems to be strongly dependent on the C mineralization rate. In fact, wetter sites showed lower productivity than the D-FR site (the most productive), but had a much slower organic C mineralization rate (see Table 4). The negative correlation between C mineralization rate and precipitation ($r=-0.87$; $p<0.05$) suggests that the turnover of SOM in drier soils is faster than in wetter soils, a trend also reported by Franzluebbers et al. (2001) for contrasting climatic conditions in North America and by Garcia-Pausas et al. (2008) for Spanish mountain grasslands. Moreover, the metabolic quotient tended to be higher in drier sites, indicating that their soil microbial communities are less efficient in C utilization.

Despite low mean annual precipitation, the D-FR site showed higher productivity than the others, which seems to be associated with high atmospheric humidity in the former (mean for 30-yr period: 80%; Reis and Gonçalves, 1981), and it may contribute to reduce the impacts of cold winters and summer drought (Jones et al. 1999). The climate conditions at the D-FR site (with high atmospheric humidity in summer and narrow annual temperature range) may favour eucalyptus productivity, SOM decomposition and C mineralization rate. Our

results agree with the high decomposition rate reported by Magalhães (2000) for eucalyptus harvest residues in the D-FR site, as compared with sites similar to the D-PR site, and also agree with the higher decomposition rate measured at the D-FR than at inland sites reported by Berg et al. (1993). Likewise, the high soil C mineralization rate in the D-FR site is in agreement with the negligible effect of slash retention on soil C contents previously obtained at the same plantation (Madeira et al. 2004).

Table 6 Simple linear regression (r) between N mineralization (mg N kg⁻¹ soil d⁻¹) and mean annual precipitation (MAP) and soil properties (n=5)

	Net mineralization ¹ (anaerobic)	Net mineralization ² (aerobic)	Net nitrification ² (aerobic)	Gross mineralization ³
MAP	0.91*	0.89*	0.88*	0.86*
Corg	0.96**	0.98**	0.98**	0.94*
Cmic	0.89*	0.98**	0.97**	0.99***
Ntotal	0.98**	0.92*	0.93*	0.90*
Nmic	0.89*	0.98**	0.97**	0.95*
NH ₄ -N	0.72	0.90*	0.90*	0.78
Ext P	0.67	0.87*	0.87*	0.94*

* Significant at $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$

¹ Incubation: 2 weeks. ² Incubation: 32 weeks. ³ Incubation: 1 day.

Net N mineralization

Net N mineralization increased in the studied gradient from drier to wetter sites, which is in agreement with results reported by Adams and Attiwill (1986) for Australian eucalypt forests covering a wide range of precipitation (570-1500 mm). The differences observed were related to an enhancement in substrate availability in wetter sites, because positive correlations were obtained between net N mineralization determined by anaerobic and aerobic incubations and initial contents of total C and N, NH₄-N and most labile fractions (see Table 6). Net N mineralization also increased in sites rich in extractable P, indicating that there is a strong relationship between the availability of these two main nutrients that limit eucalyptus growth in Portugal (Pereira et al. 1996). This is in agreement with the stimulation of N mineralization following P addition in Australian eucalypt and pine forests reported by Falkiner et al. (1993).

While the amount of N mineralized in both anaerobic and aerobic incubations was highest in the wettest site (W-CS), the highest N mineralization rates were observed in the driest D-FR site, indicating a fast N cycling in this low N-status soil. The high rates of N anaerobically mineralized (Table 5) and the predominance of net ammonification during the first weeks of aerobic incubation (Figure 2) suggest that such N availability occurs mainly as NH₄-N.

Cumulative net ammonification declined and net nitrification increased as the length of the incubation increased, possibly due to the depletion of readily available substrate because in biogeochemical cycles the most oxidized state of an element (in this case nitrate) only occurs if there is a lack of available energy, i.e., organic carbon (Verhagen et al. 1995). Also, studying a forest soil, Hart et al. (1994) concluded that a decrease in C availability could cause a relative change in nitrification and nitrate immobilization, which could explain the lag in

net nitrification observed during long-term soil incubations. This is in agreement with the positive correlation found in the present study between the decline in the C_{POM} contents during aerobic incubation and the cumulative amount of net nitrification after 32 weeks ($r=0.93$, $p=0.02$). This trend was not observed for hot water and hydrolizable C contents, which either

did not change or increased during incubation (Table 3). This suggests that the particulate organic matter fraction may be the major source of C for forest microorganisms in the soils studied, such as reported by Hart et al. (1994) for the reduction of extractable organic pool during a long-term incubation.

Fig. 2 Cumulative net ammonification (a) and net nitrification (b) during 32 weeks of aerobic incubation. Different letters for data at the end of the incubation indicate significant differences among means ($n=4$) at the $P = 0.05$ level.

Patterns of net nitrification differed among the studied plantations. A rapid nitrate accumulation was found in the wettest site (W-CS), while in drier sites the lag time before the beginning of nitrate production was longer. The cumulative nitrification after 8-weeks at the D-FR site was similar to those previously reported for the same plantation during similar laboratory incubations period (Madeira et al. 2004), but our results indicate that nitrification became the dominant process when a longer period is considered (e.g. 16-weeks). A lack of nitrification was also observed for soil laboratory incubations (10-weeks) of eucalypt forest under Mediterranean climate (Adams and Attiwill 1986) and for *in vitro* soil incubations (3-weeks) of tropical eucalypt plantations (Bernhard-Reversat 1996). For Australian eucalypt forest soils, Attiwill et al. (1996) suggested that net nitrification measured by *in situ* incubations is predominant for a C/N ratio lower than 15 (Attiwill et al. 1996), which support our results, as net ammonification was the dominant process during the first 4-weeks of incubation. However, this threshold could be

wider, as ammonification has been found to predominate during *in situ* incubations (50 days) carried out in eucalypt plantations with low (12) soil C/N ratio (Gonçalves et al. 1999), while nitrification has been reported for eucalypt soils with C/N ratio of 18 (Moroni et al. 2002). Other factors could also be involved in the soil nitrification process, such as previous soil use, understory species or soil texture. For example, Bernhard-Reversat (1996) reported different nitrification patterns in clay and sandy eucalypt soils developed under similar conditions. The presence of leguminous species in the understory of the W-CS site (*Pterospartum tridentatum*) may also be associated with an enhancement of soil nitrification, as observed for experimental eucalyptus growing with N-fixing species (Khanna 1998; Garcia-Montiel and Binkley 1998).

The cumulative net nitrification after 32-weeks increased in sites initially rich in total and labile C and N, NH_4 -N and extractable P (see Table 6), suggesting that differences in net nitrification among studied sites might be due to a higher substrate availability in the wetter sites.

In the most productive plantations, eucalypt tree root competition for $\text{NH}_4\text{-N}$ could have reduced the substrate availability, leading to low nitrification during the laboratory incubation. In fact, nitrification was the dominant process in a lysimeter experiment without trees over 6 years using soils from the D-FR site (Gómez-Rey et al. 2007), which suggests that autotrophic nitrifiers are relatively inactive under field conditions and

the absence of tree root competition in lysimeters may lead to their proliferation. This is supported by the results of a laboratory incubation experiment (Gómez-Rey, unpublished data), in which the inoculation of nitrifying soil and the addition of $\text{NH}_4\text{-N}$ to soils from the same site duplicated the amount of nitrate produced after 24-weeks of aerobic incubation.

Fig. 3 Gross N mineralization, $\text{NH}_4\text{-N}$ immobilization, nitrification and $\text{NO}_3\text{-N}$ immobilization. Error bars refer to standard errors and different letters indicate significant differences among means ($n=6$) of the same variable at the $P= 0.05$ level

Gross N mineralization

Amounts of gross mineralization and nitrification observed in the present study were within the range reported for Australian eucalypt plantations (Livesley et al. 2007) and for other forestry species from Australia (Burton et al. 2007) and from eastern (Verchot et al. 2001) and western (Hart et al. 1997) North America. However, gross N mineralization was relatively low compared with that reported for eucalyptus plantations in Hawaii (Garcia-Montiel and Binkley 1998), probably related to the high amount of C of those allophanic soils. Values of daily gross mineralization were higher than those of net mineralization measured by anaerobic and aerobic incubation (2 and 19 times, respectively). Despite this difference, results of the isotope dilution technique supported the conclusions from net N flow measurements, with higher amounts of N mineralized in the wettest site (W-CS) but faster turnover in the driest sites (D-PG and D-FR, see Table 4). Moreover, net and gross mineralization were generally correlated with the same factors (Table 6). The fact that gross N mineralization was positively correlated with net mineralization determined by long- (32 weeks) or medium-term (16 weeks) aerobic incubation ($r=0.95$, $P=0.012$; $r=0.92$, $P=0.031$, respectively), but not with short-term (2 weeks) aerobic ($r=0.83$, $P=0.077$) and anaerobic incubation ($r=0.85$, $P=0.064$), suggests that net measurements could be an index of differences on N cycling patterns in these soils. Therefore, at least a medium-term aerobic incubation period is necessary to be within the period with correlation between gross N mineralization and net mineralization.

However, as reported by several authors (Stark and Hart 1997, Davidson et al. 1992), our study suggests that net nitrification measurements did not predict gross nitrification. Indeed, gross nitrification and cumulative net nitrification (after 2, 16 or 32 weeks) are not significantly correlated and factors that mediate the patterns of net nitrification did not influence gross nitrification (see Table 6). Thus, lack of net nitrification did not always coincide with the absence of gross nitrification. For example, in the driest sites (D-PR and D-FR) net nitrification did not occur during 2 weeks of aerobic incubation, but gross nitrification was detected after 1 day of

incubation, indicating that $\text{NO}_3\text{-N}$ production in these soils is a more important process than would be expected from net nitrification measurements.

In all studied sites, the $\text{NO}_3\text{-N}$ produced was rapidly assimilated by microorganisms, as gross $\text{NO}_3\text{-N}$ immobilization always exceeded gross nitrification. Previous studies have shown the importance of $\text{NO}_3\text{-N}$ immobilization in forest soils (Davidson et al. 1992; Hart et al. 1994; Stark and Hart 1997), suggesting that large amounts of $\text{NO}_3\text{-N}$ can be produced internally in these systems without subsequent nitrogen loss. This was more evident in the low N content D-PG site, where both gross and net nitrification were not detected in the short-term but $\text{NO}_3\text{-N}$ immobilization was observed. Similar results were reported by Garcia-Montiel and Binkley (1998) in eucalyptus soils from Hawaii, who concluded that these soils showed a high capacity to immobilize any form of inorganic N present when N is limiting.

Conclusions

Soil C and N contents, both total and labile, and amounts of C and N mineralized increased within the precipitation gradient. Despite their low soil C and N status, the driest sites were characterized by high turnover of C and N, which may affect eucalypt productivity. Patterns of net and gross N mineralization were similar, but isotope dilution techniques are necessary to reveal the relative importance of the nitrification process. The particulate organic matter fraction may be the most sensitive indicator of differences in soil C and N dynamics of the soils studied, as only this labile fraction was related to C and net N mineralization determined by long-term incubation. Our results suggest that future changes in precipitation, which are predicted to be significant in Mediterranean areas, might influence the stocks of total and labile C. As a consequence of their important role in N cycling, amounts of N mineralized and nitrified may be also affected.

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