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Article

Available Nitrogen and Responses to Nitrogen Fertilizer in Brazilian Eucalypt Plantations on Soils of Contrasting Texture

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Abstract: *Eucalyptus* plantations have seldom responded to N fertilization in tropical and subtropical regions of Brazil. This implies that rates of N mineralization have been adequate to supply tree needs. However, subsequent crop rotations with low N fertilization may result in declining concentrations of organic and potentially mineralizable N (N_0), and consequent loss of wood productivity. This study investigated (a) *in situ* N mineralization and N_0 in soils of eucalypt plantations in São Paulo state, Brazil; (b) tree growth responses to N fertilizer applied 6–18 months after planting; and (c) the relationships between N_0, other soil attributes and tree growth. We established eleven N fertilizer trials (maximum 240 kg ha^{-1} of N) in *E. grandis* and *E. grandis* x *urophylla* plantations. The soil types at most sites were Oxisols and Quartzipsamments, with a range of organic matter (18 to 55 g kg^{-1}) and clay contents (8% to 67%) in the 0–20 cm layer. Concentrations of N_0 were measured using anaerobic incubation on soil samples collected every three months (different seasons). The samples collected in spring and summer had N_0 140–400 kg ha^{-1} (10%–19% total soil N), which were best correlated with soil texture and organic matter content. Rates of *in situ* net N mineralization (0–20 cm) ranged from 100 to 200 kg ha^{-1} year^{-1} and were not correlated with clay, total N, or N_0. These high N mineralization rates resulted in a low response to N fertilizer application during the early ages of stand growth, which were highest on sandy soils. At the end of the crop rotation, the response to N fertilizer was negligible and non-significant at all sites.

Keywords: forest; productivity; potentially mineralizable N; sustainability; fertilizer application; nutrition

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1. Introduction

Establishment of a forest plantation using the *Eucalyptus* genus for pulp and paper production and other products is justified by their high productivity across different soils and climates. However, sustainable wood production might be compromised in the short- or long-term when plantations are established on soils of low fertility, such as Oxisols and Quartzipsamments [1,2]. Productive plantations with high capacity for nutrient extraction can greatly impact N pools in the soil. Adequate management practices, including fertilizer application, are required to sustain tree growth rates and ensure soil quality over successive rotations [3].

Although trees require large amounts of N, some researchers found that eucalypts did not respond to N fertilizer application under tropical and subtropical climate conditions [1,4,5]. Commonly, N fertilizer application enhances the early growth of a eucalypt stand, but the response is not continued through the entire rotation [3–5]. The lack of response possibly occurs due to significant mineralization rates of organic N [3,6] and to atmospheric N deposition [7–10]. Mineralization of organic N is the main natural N source for plantations and appears to be sufficient to meet tree demand [3,11], which in southeast Brazil ranges from 20 to 50 kg ha^{-1} year^{-1} [3,6]. However, intensively managed eucalypt plantations are expected to respond to N fertilizer application following several crop rotations because of high N outputs via harvesting [3,6,12], low N fertilizer rates applied [1,13], and depletion of organic N pools in soil [3]. Some studies carried out at sites with low soil organic matter (SOM) and N status showed that eucalypt stands might respond to N application under this condition [14,15]. Hence, the need for N fertilization in Brazilian plantations should be examined each rotation across a range of soil types.

The complexity of determining N fertilizer recommendations is attributed to the difficulty in accurately predicting the supply of available N (N mineralization). Gonçalves *et al.* [2] described a recommendation index for N fertilizer application to eucalypt plantations in Brazil based on SOM content. Although SOM is the main source of N, such an indicator does not consider the effect of SOM quality, climate and forest management [16,17].

The definition of a useful index of N availability is one that is practical, and chemical analyses can be faster than biological assays [18,19]. Several laboratory methods have been proposed to estimate potentially mineralizable N (N_0) in soils, and the anaerobic incubation method appears promising [11,20,21].

To better understand the interactions of N supply and tree growth at this stage of eucalypt plantation development in southeast Brazil,, this study investigated (a) *in situ* N mineralization and N_0 in soils of eucalypt plantations in São Paulo state, Brazil, (b) tree growth responses to N fertilizer applied 6–18 months after planting, and (c) the relationships between N_0, other soil attributes and tree growth.

2. Material and Methods

2.1. Site Description

We selected eleven *Eucalyptus grandis* and *Eucalyptus grandis* × *urophylla* sites in São Paulo state, Brazil, ranging from 1 to 11.4 years old. The sites belong to industrial companies and to the Itatinga Experimental Station of Forest Sciences, University of São Paulo and are representative of the majority of soils, climates and plantation management in this state (Figure 1). The climate is Cwa, according to Köppen classification at Altinópolis site; Cfa at Agudos, Angatuba, Capão Bonito, São Miguel Arcanjo and Votorantim sites; and Cfb at Botucatu, Itatinga and Paraibuna sites [22]. Cwa is a humid subtropical climate with a dry winter and a hot summer. Cfa is a humid subtropical climate without a dry season and with a hot summer. Cfb is a humid subtropical climate without a dry season and with a temperate summer. C climate types have an average annual temperature below 18 °C. Cw climates have rainfall of the driest month (R_{DRY}) more than 40 mm, while the Cf climates have R_{DRY} less than 40 mm. Average annual rainfall at the studied sites ranges from 1170 to 1517 mm (Table 1). Soils in the municipalities of Itatinga, São Miguel Arcanjo and Paraibuna are Typic Hapludox (Red-Yellow Latosol); at Altinópolis, Angatuba and Botucatu, Typic Quartzipsamment (Quartzarenic Neosol); at Agudos and Capão Bonito 2, Typic Hapludox (Red Latosol); at Capão Bonito 1, Typic Hapludox (Yellow Latosol); at Votorantim, Typic Paleudult (Red-Yellow Argisol); and at Capão Bonito 3, Typic Dystropept (Dystrophic Cambisol) [23]. These are the main soils used in forest plantations in São Paulo State [2]. Further soil details of Itatinga and Capão Bonito can be found in Gonçalves *et al.* [24] and Alvares *et al.* [25], respectively. The contents of SOM ranged from 18 to 55 g kg^{-1} and clay contents from 8% to 68% in the 0–20 cm layer (Table 2). They are also quite acidic with pH 3.9–4.9.

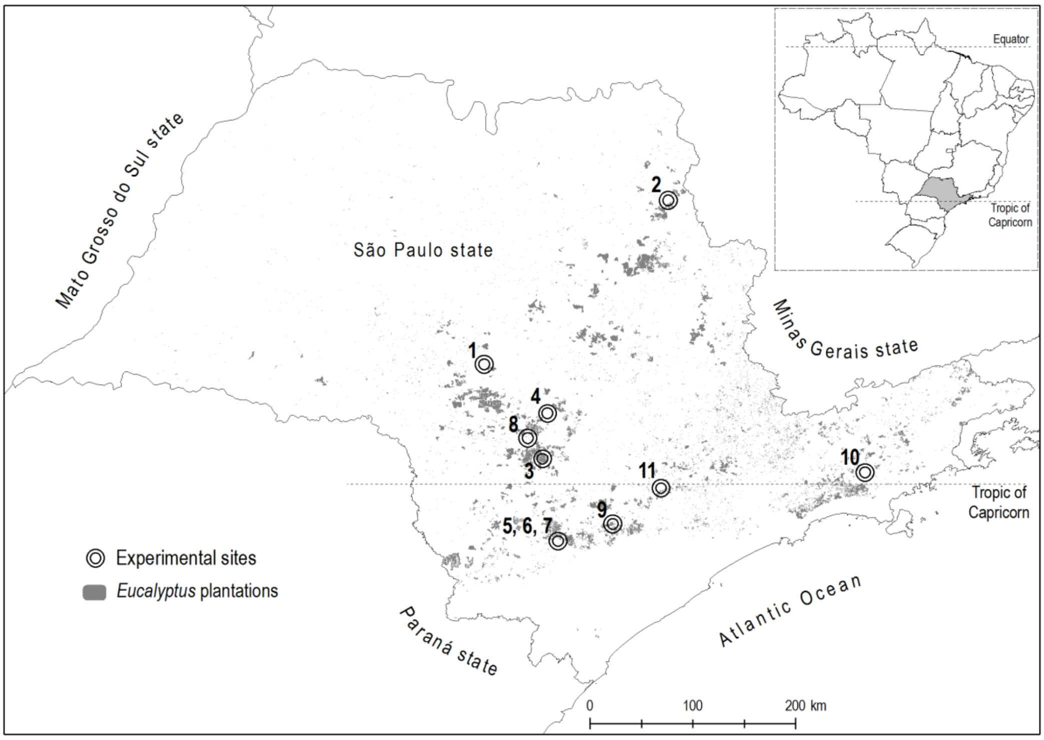

Figure 1. Map of São Paulo state showing locations of the experimental sites: 1—Agudos; 2—Altinópilis; 3—Angatuba; 4—Botucatu; 5, 6, 7—Capão Bonito; 8—Itatinga; 9—São Miguel Arcanjo; 10—Paraibuna; and 11—Votorantim.

At each site, we used a randomized block experimental design with three replicates. Measured plots were 10 m by 10 m, with 36 trees per plot, surrounded by a two-tree border. The treatments were: (i) control (low N dose of fertilizer application, sufficient to provide good establishment of plants; *c*. 15 kg ha^{-1}); (ii) usual dose (N dose used by the companies; *c*. 100 kg ha^{-1}); and (iii) increased dose (100% higher than commercial doses; *c*. 200 kg ha^{-1}). The N fertilizer application schedule was 25% of total dose at planting, and the rest applied equitably at 6, 12 and 18 months after planting. The fertilizer application after planting was placed in a continuous line under canopy projection. Additional to N fertilization, all treatments received 30 kg ha^{-1} of P, 130 kg ha^{-1} of K, 230 kg ha^{-1} of Ca, 100 kg ha^{-1} of Mg, 3 kg ha^{-1} of B, 60 kg ha^{-1} of S, 1.5 kg ha^{-1} of Zn and 0.5 kg ha^{-1} of Cu. Calcium and Mg were supplied through lime application. Throughout the experiment, the stands were kept free of weed competition by herbicide application.

Table 1. Latitude (Lat), longitude (Long), altitude (Alt), mean annual temperature (T), mean annual pluviometric precipitation (PP), topography, planted genotype, tree spacing and planting date of each experiment.

Municipality	**Site Code**	**Lat**	**Long**	**Alt**	**T** [3]	**PP**	**Geology** [4]		**Genotype**	**Tree Spacing**	**Planting**
							Formation or Group	**Lithotype**			
		S	W	m	°C	mm				m	
Agudos	AGU	22°28′	48°59′	580	20.6	1170	Marília	Sandstone, sandy argillite and limestone	*E.grandis* [1]	3.0 × 2.0	Aug-2005
Altinópolis	ALT	21°01′	47°22′	889	19.4	1517	Botucatu	Quartz-sandstone	*E.grandis* × *urophylla* [2]	3.0 × 2.5	May-2002
Angatuba	ANG	23°17′	48°28′	649	19.7	1262	Pirambóia	Shale, thin sandstone and silty-clayey sandstone	*E.grandis* × *urophylla* [2]	3.0 × 2.0	Apr-2006
Botucatu	BOT	22°53′	48°26′	804	19.1	1302	Marília	Sandstone, sandy argillite and limestone	*E.grandis*[1]	3.0 × 2.0	Nov-2005
Capão Bonito 1	CB1	24°00′	48°20′	705	18.9	1210	Itararé	Sandstone, diamictite and shale	*E.grandis* × *urophylla* [2]	3.0 × 3.0	Jun-1999
Capão Bonito 2	CB2	24°00′	48°20′	705	18.9	1210			*E.grandis* × *urophylla* [2]	3.0 × 2.0	Feb-2007
Capão Bonito 3	CB3	24°00′	48°20′	705	18.9	1210			*E.grandis* × *urophylla* [2]	3.0 × 2.0	Dec-2006
Itatinga	ITA	23°06′	48°36′	845	18.8	1308	Marília	Sandstone, sandy argillite and limestone	*E.grandis*[1]	3.0 × 2.0	Apr-2002
São M. Arcanjo	SMA	23°51′	47°51′	715	18.9	1174	Itararé	Sandstone, diamictite and shale	*E.grandis* × *urophylla* [2]	3.0 × 2.0	Aug-2006
Paraibuna	PAR	23°23′	45°39′	634	19.2	1249	Natividade da Serra	Monzogranite, biotite and granite	*E.grandis*[1]	3.0 × 2.5	Mar-1997
Votorantim	VOT	23°32′	47°26′	570	19.8	1287	Granite Sorocaba	Granite, Granodiorite, monzogranite and syenogranite	*E.grandis* × *urophylla* [2]	3.0 × 2.0	Oct-2006

[1] Seedling plantations; [2] Clonal plantations; [3] Alvares *et al.* [26]; [4] IPT [27].

Table 2. Soil physical and chemical attributes (0–20 cm layer) at the eleven sites.

Site	Clay [1]	Silt [1]	Sand [1]		OM [2]	pH [3]	P-resin [4]	Cation Exchange [4]			
			Coarse	Fine				K	Ca	Mg	Al
	%				g kg^{-1}		mg kg^{-1}	$\text{mmol}_c\ \text{kg}^{-1}$			
AGU	16.7	2.7	30.7	49.9	15	3.9	2.3	0.6	1.0	0.6	8.3
ALT	6.7	1.3	38.7	53.3	13	4.3	4.4	0.8	4.8	1.9	3.7
ANG	10.0	1.0	29.3	59.7	16	4.0	8.5	0.5	5.3	1.5	7.6
BOT	10.0	3.0	32.0	55.0	11	4.0	4.8	0.3	3.0	3.7	4.1
CB1	47.8	10.4	8.7	33.1	23	3.9	2.2	0.8	1.0	0.8	12.8
CB2	65.3	15.3	5.3	14.1	29	4.4	3.6	1.6	4.4	4.8	13.7
CB3	27.2	23.4	1.0	48.4	16	4.1	3.7	1.8	2.7	2.6	19.1
ITA	19.3	2.2	37.5	41.0	18	4,0	2.3	0.6	1.6	1.8	9.7
SMA	65.1	17.3	2.9	14.7	45	4.9	46.9	5.3	48.6	13.6	1.3
PAR	36.5	5.5	43.9	14.1	15	4.1	3.6	2.0	29.0	10.3	0.9
VOT	67.0	11.1	15.3	6.6	46	4.0	4.2	1.6	1.8	1.0	14.7

[1] Pipette method [28]; [2] Organic matter determined by potassium dichromate and sulfuric acid extraction; [3] $CaCl_2$ 0.01mol L^{-1} soil to solution ratio 1:2.5; [4] Ion Exchange resin [29].

2.2. Growth Assessment

In all plots, we measured diameter at breast height (DBH), total height and tree survival, and estimated the mean annual increment (MAI) of solid wood volume (SV) with bark. The SV was estimated by allometric equations using DBH and height developed by companies specifically for the genetic material used in each plantation. To compare eucalypt response to N fertilizer application in all sites, regardless of the growth increment, relative productivity (RP) increase was calculated as follows (Equation (1)).

$$\text{RP (\%)} = \frac{\text{SV}}{\text{SVmax}} \times 100 \tag{1}$$

where: "SV" is solid wood volume with bark of a given treatment and "SVmax" is the solid wood volume with bark of the highest dose treatment.

2.3. Soil Analysis

Soil chemical and physical properties were determined for the 0–20 cm layer at all sites. Ten soil samples were collected from each plot, in a diagonal transect across the inner part of the plot between planting lines. The samples were used to make one mixed sample per plot, which was air dried and homogenized. Next, the samples were sieved through a 2 mm mesh and mixed up again.

Particle size analysis (pipette method) of soil was performed according to Embrapa [28]. We determined the pH in $CaCl_2$ 0.01 mol L^{-1}, available phosphorus and exchangeable calcium, magnesium, potassium extracted by ion-exchange resins and aluminum extracted by KCl 1 M, according to Raij *et al.* [29]. We assessed soil total carbon (C_t) at all sites in the control treatment (0–20 cm layer) using the South Dakota method with modifications by Raij *et al.* [29]. This method consists of organic matter oxidation by dichromate ($K_2Cr_2O_7 + H_2SO_4$), and quantification by colorimetry. Total N (N_t) was determined by the micro-Kjeldahl method [30].

2.4. Assessment of N Mineralization

At all sites, soil samples from the 0–20 cm layer were collected to evaluate potential N mineralization under anaerobic conditions in the laboratory (N_0). The samples were collected in April (fall), July (winter), November (spring), 2007, and in January (summer), 2008. We performed anaerobic incubations (40 °C for 7 days) using chemical methods according to those proposed by Keeney and Bremner [20]. Prior to incubation, 30 mL of nutritional solution, consisting of: $MgSO_4$ (0.002 mol L^{-1}) and Ca $(H_2PO_4)_2$ (0.005 mol L^{-1}), was added. Jars were manually shaken until soil dispersion, and then covered with polyethylene film to avoid water loss by evaporation and algal growth [11]. N was extracted again 7 days after incubation by adding 4.47 g of KCl to each jar, which provide a concentration of 2 mol L^{-1} of KCl. Jars were then shaken manually for about 60 s, placed to rest 24 h and then filtered and analyzed for NH_4 as described above. To calculate potentially mineralizable N we subtracted the initial concentrations of mineral N.

To measure net N mineralization, the *in situ* method of Raison *et al.* [31] was used to in the 0–20 cm soil layer of control plots. Five pairs of steel tubes 30 cm long and 5 cm diameter were placed between tree rows in a diagonal transect across the inner part of the plot. The principle of the method is that inserting a sharpened corer severs roots, and adding capping during *in situ* incubation excludes rainfall. Net N mineralization thereby proceeds without leaching or uptake. By measuring mineral N initially (in additional representative soil samples) and again after incubation, net ammonification and nitrification can be calculated. For this study, one tube of each pair was immediately removed to assess initial mineral N concentrations (t_0) for all plots, and the remaining tubes were covered to avoid leaching of N, remaining in soil for 30 days on average. The amount of mineral N at t_0 was subtracted from the mineral N content after incubation to calculate *in situ* net N mineralization for each 30 day period. The same rate was assumed for other periods of the same season. Soil from each set of five tubes per plot was mixed to obtain a composite sample. The *in situ* method was used at all sites for January–December 2008.

For each collection, samples at all sites were collected within 8 days of each other to minimize time-related climatic variations between sites. The tubes were transported vertically to the lab, to minimize disturbance, and they were kept in thermal boxes (2–5 °C), individually wrapped in plastic bags. Refrigeration was used to minimize microbial activity, reducing mineralization that could occur prior to mineral N extraction [32]. Soil samples remained refrigerated until extraction, which was performed within two days of each collection.

For initial N (t_0) extraction, 10 g of soil were placed in 110 mL jars and 100 mL of KCl 2 mol L^{-1} added. Jars were manually shaken for *c*. 60 seconds for soil dispersion, and placed to rest for 24 h. Afterwards, the suspension was filtered through Whatman No. 42 filter paper, and the filtrate was analyzed to for NH_4^+ and NO_3^-, following the addition of 0.1 mL of microbial inhibitor (mercury acetate phenyl 0.5 mg L^{-1}).

For both N_0 and *in situ* N mineralization, NH_4^+ and NO_3^- concentrations were determined using the Analyze System of Injection in Automatic flow—ASIA (Ismatec, Glattbrugg, Switzerland) [33] and detected by colorimetry at 605 nm, which has a detection limit of 0.01 μg mL^{-1}.

2.5. Data Analysis

Data were checked for normality (Shapiro-Wilk) and homoscedasticity (Box-Cox). ANOVA was used to assess the significance of differences between means. Where there was a significant ($p < 0.05$) F test, a Tukey test (5% probability) was used for contrasting means. The relationship between dependent and independent variables was assessed by Pearson correlation and linear regression.

3. Results

Net N mineralization *in situ* rates ranged from 4.8 to 11.5 kg ha^{-1} month^{-1} (fall and winter) and from 10.6 to 15.0 kg ha^{-1} month^{-1} (spring and summer) for sandy soils, from 7.4 to 15.2 kg ha^{-1} month^{-1} (fall and winter) and from 11.0 to 17.6 kg ha^{-1} month^{-1} (spring and summer) for loamy soils, and from 3.6 to 19.1 kg ha^{-1} month^{-1} (fall and winter) and from 9.4 to 24.3 kg ha^{-1} month^{-1} (spring and summer) for clayey soils (Table 3). The average rates of N mineralization were highest in clayey soils, ranging from 110 to 207 kg ha^{-1} year^{-1}, and the lowest to sandy soils, ranging from 107 to 140 kg ha^{-1} year^{-1}. The average ratio of N-NH_4^+/N-NO_3 was 1.6 for sand soils, 1.7 for loamy soils and 1.9 for clayey soils.

Values of N_0 during summer ranged from 60 to 154 mg kg^{-1} of soil (190 and 398 kg ha^{-1}), with an average of 97 ± 11 mg kg^{-1} of soil (241 ± 18 kg ha^{-1}) (Table 4). Values of N_0 in sandy soils during summer were on average 168 kg ha^{-1} of N_0, 212 kg ha^{-1} in loamy soils, and 303 kg ha^{-1} in clayey soil. During winter, these values ranged from 20 to 91 mg kg^{-1} of soil (63 and 178 kg ha^{-1}). In sandy soils, N_0 corresponded to 19% of N_t, 14% in loamy soil, and 13% in clayey soils.

At all sites, tree survival was higher than 95%. On average, nitrogen fertilizer application resulted in an increase in MAI of 14% at early age, 6% at intermediate age and 0% at the end of the crop rotation (Table 5). Relative production (RP) at early age of control treatment ranged from 74% to 98% (average of 87% ± 2%), and from 90% to 98% (average of 95% ± 1%) at intermediate age. In the treatment that received commercial fertilizer application, RP ranged from 88% to 111% (average of 100% ± 2%) at early age, and from 98% to 107% (average of 100% ± 1%) at intermediate age. After five years of age (approximate harvesting age), RP ranged from 99% to 103% (average of 102% ± 1%).

The relative wood volume response to N application was greater in sandy soils, and, in absolute terms, at soils with higher clay content (Table 5 and Figure 2). During the first two years of age, RP of the control was about 16% lower than in the treatments that received commercial fertilizer application at sandy soils, and 9% to 10% at soils with higher clay content. Greater relative response to N application at early age occurred in soils with lower N_t, N_0 and clay contents (Figure 2). At the end of the rotation, regardless of soil texture, no significant responses to N application were found.

4. Discussion

4.1. N mineralization

Higher rates of N mineralization in clayey soils are attributed to higher stocks of organic N, due to the greater net primary productivity of the ecosystem, and to higher amounts of soil organic-mineral complexes (Tables 3 and 4). Under this condition, microbial activity increases due to higher amounts of substrate [34,35] and water availability. Eaton [36] reported that after two days of intensive rains, clayey soils in subtropical forests showed a significant increase in microbial C and N-NH$_4^+$ mineralization rates, compared to sandier soils. The author speculated that part of the active organic matter pool became detached from soil clay particles, and thereby became available to microorganisms.

Low rates of nitrification (Table 3) might be caused by high acidity and low fertility in these soils, and high NH$_4^+$ uptake under high growth rate stands could restrict substrate availability for nitrifying bacteria [3,37–39]. Under high acidity and low soil fertility, nitrifying bacteria (*Nitrobacter* and *Nitrosomonas*) have impaired active. Higher NH$_4^+$ than NO$_3^-$ availability is usually not a limitation for eucalypt nutrition [40,41]. Gonçalves and Carlyle [39] studying N mineralization in *Pinus radiata* plantation soils under laboratory conditions reported that, despite the increased rate of nitrification during incubation, it was not proportional to the reduction of NH$_4^+$ concentration, showing the possibility of NH$_4^+$ immobilization and/or denitrification caused by soil moisture variations.

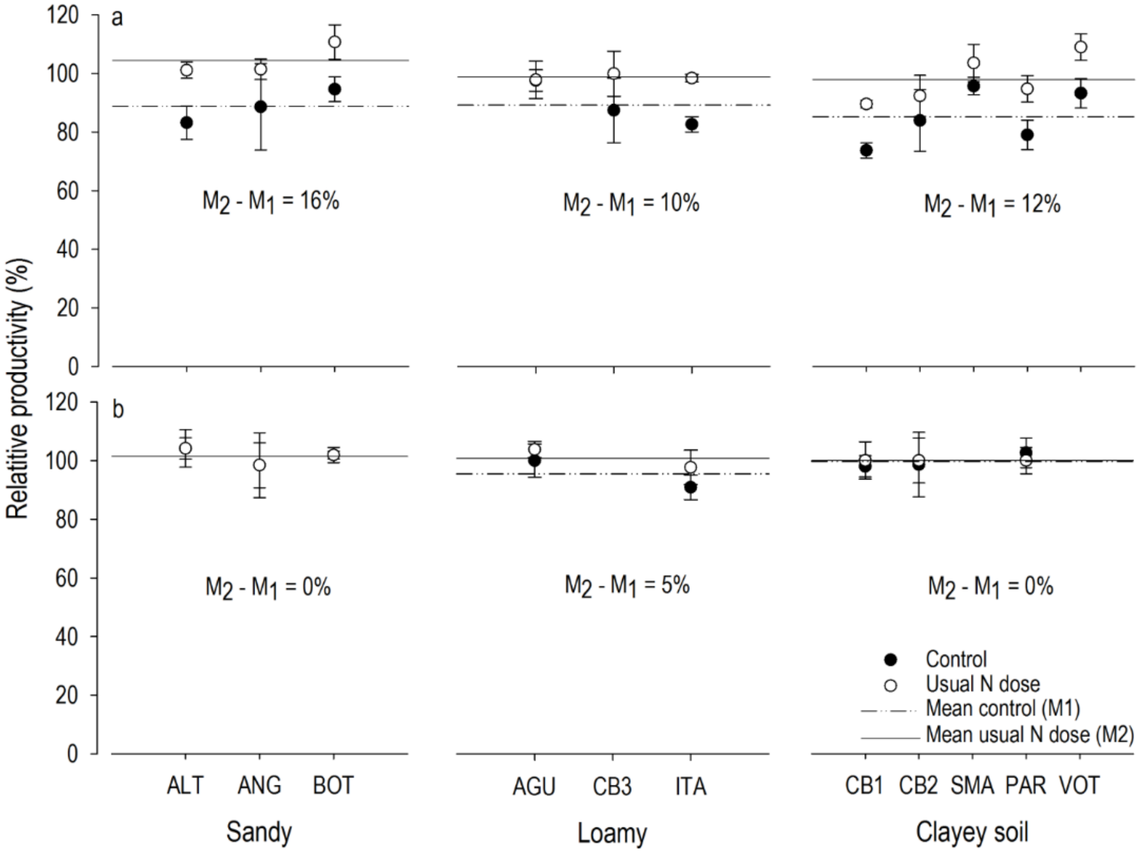

Figure 2. Relative productivity (RP) of the control treatments in relation to the usual and increased N rate treatments. (**a**) at young ages (less than two years) and (**b**) later ages (more than five years) at all sites, grouped according to soil texture. See Table 1 for *x*-axis site codes.

Table 3. Rates of net ammonification and net nitrification *in situ* and N-NH_4^+/N-NO_3^- ratio at each site (0–20 cm layer).

Site	**Summer**				**Fall**				**Winter**				**Spring**				**Yearly Total**			
	N-NH_4^+	**N-NO_3^-**	**N-total**	**NH_4^+/NO_3^-**	**N-NH_4^+**	**N-NO_3^-**	**N-total**	**NH_4^+/NO_3^-**	**N-NH_4^+**	**N-NO_3^-**	**N-total**	**NH_4^+/NO_3^-**	**N-NH_4^+**	**N-NO_3^-**	**N-total**	**NH_4^+/NO_3^-**	**N-NH_4^+**	**N-NO_3^-**	**N-total**	**NH_4^+/NO_3^-**
	kg ha^{-1} $month^{-1}$				kg ha^{-1} $month^{-1}$				kg ha^{-1} $month^{-1}$				kg ha^{-1} $month^{-1}$				kg ha^{-1} yr^{-1}			
Sandy Soils																				
ALT	5.7	8.0	13.7	0.7	4.6	4.1	8.7	1.1	6.6	2.6	9.2	2.6	10.1	4.8	15.0	2.1	81.2	58.6	139.8	1.4
ANG	8.6	3.8	12.4	2.3	3.3	1.6	4.8	2.1	4.3	1.3	5.5	3.4	8.0	5.1	13.1	1.6	72.4	35.2	107.6	2.1
BOT	5.6	7.6	13.2	0.7	6.9	4.5	11.5	1.5	4.1	3.7	7.8	1.1	9.1	1.5	10.6	6.1	77.0	52.0	129.0	1.5
Mean	6.6	6.5	13.1	1.2	4.9	3.4	8.3	1.6	5.0	2.5	7.5	2.4	9.1	3.8	12.9	3.3	76.9	48.6	125.5	1.6
s [1]	1.7	2.3	0.7	0.9	1.8	1.6	3.4	0.5	1.4	1.2	1.9	1.2	1.1	2.0	2.2	2.5	4.4	12.1	16.4	0.4
Loamy Soils																				
AGU	11.2	6.5	17.6	1.7	9.0	4.2	13.2	2.2	6.1	3.2	9.3	1.9	11.3	3.2	14.5	3.5	113.0	51.1	164.0	2.2
CB3	6.9	7.5	14.4	0.9	3.9	5.9	9.8	0.7	6.8	4.9	11.7	1.4	8.0	3.0	11.0	2.7	76.8	63.7	140.5	1.2
ITA	8.5	4.4	12.9	2.0	3.3	4.1	7.4	0.8	9.2	6.1	15.2	1.5	9.6	3.0	12.6	3.2	91.7	52.6	144.3	1.7
Mean	8.9	6.1	15.0	1.5	5.4	4.7	10.1	1.2	7.4	4.7	12.1	1.6	9.6	3.1	12.7	3.1	93.8	55.8	149.6	1.7
s	2.2	1.6	2.4	0.6	3.1	1.0	2.9	0.8	1.6	1.5	3.0	0.3	1.7	0.1	1.8	0.4	18.2	6.9	12.6	0.5
Clayey Soils																				
CB1	7.0	1.9	8.9	3.7	8.9	6.7	15.6	1.3	4.0	8.6	12.6	0.5	13.0	6.8	19.8	1.9	98.7	72.2	170.9	1.4
CB2	11.2	4.9	16.0	2.3	3.6	2.3	5.9	1.5	3.8	7.0	10.9	0.5	11.8	5.3	17.1	2.2	91.1	58.7	149.8	1.6
SMA	6.0	3.4	9.4	1.8	6.5	1.2	7.7	5.4	3.4	1.9	5.2	1.8	9.7	4.7	14.4	2.1	76.9	33.5	110.4	2.3
PAR	14.3	7.8	22.1	1.8	12.7	6.5	19.1	1.9	1.1	2.6	3.6	0.4	17.7	6.6	24.3	2.7	137.2	70.2	207.4	2.0
VOT	13.4	6.9	20.4	1.9	6.4	2.6	9.0	2.5	7.5	1.9	9.4	3.9	10.2	3.5	13.8	2.9	112.5	45.0	157.5	2.5
Mean	10.4	5.0	15.4	2.3	7.6	3.9	11.5	2.5	3.9	4.4	8.3	1.4	12.5	5.4	17.9	2.4	103.3	55.9	159.2	1.9
s	3.7	2.4	6.1	0.8	3.4	2.6	5.6	1.7	2.3	3.2	3.8	1.5	3.2	1.4	4.3	0.4	22.9	16.6	35.1	0.5

[1] Mean standard deviation.

Table 4. Seasonal values of potentially mineralizable N (N_0), total C (C_t), total N (N_t), C/N ratio and N_0/N_t ratio at each site grouped by texture class.

Site [1]	N_0								C_t [2]	N_t [3]	N_0 [4]	N_0/N_t	C/N
	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer					
	mg kg^{-1}				kg ha^{-1}				mg kg^{-1}			%	
Sandy Soils													
ALT	26	20	47	60	82	63	149	190	7723	387	60	15	20
ANG	28	46	39	61	63	103	88	137	7025	323	61	19	22
BOT	53	31	56	66	142	83	150	177	7090	301	66	22	24
Mean	36	32	47	62	96	83	129	168	7279	337	62	19	22
s [5]	15.0	13.1	8.5	3.2	41.2	20.0	35.5	27.6	385.6	44.7	3.2	3.5	2.0
Loamy Soils													
AGU	30	42	73	60	84	118	205	169	9233	387	60	16	24
CB3	104	– [6]	95	115	279	–	255	309	9342	810	115	14	12
ITA	71	43	73	75	149	90	153	158	7850	589	75	13	13
Mean	68	43	80	83	171	104	205	212	8808	595	83	14	16
s	37.1	0.7	12.7	28.4	99.3	19.8	51.0	84.2	831.7	211.6	28.4	1.5	6.7
Clayey Soils													
CB1	56	69	91	111	139	171	226	275	13,403	774	111	14	17
CB2	114	–	112	107	276	–	271	259	18,814	981	107	11	19
SMA	34	91	98	125	67	178	192	245	24,498	1267	125	10	19
PAR	125	83	108	138	361	240	312	398	10,837	893	138	15	12
VOT	158	–	134	154	344	–	292	336	29,724	1276	154	12	23
Mean	97	81	109	127	237	196	258	303	19,455	1038	127	13	18
s	51.1	11.1	16.4	19.4	129.3	38.0	49.0	63.6	7781.3	225.3	19.4	2.1	4.0

[1] Described in Table 1; [2] Determined by humid oxidation; [3] Bremmer [30] method; [4] adaptation of Keeney and Bremner [20] method; [5] Mean standard deviation; [6] Data not collected.

Gonçalves *et al.* [11] found similar values of N_0 in eucalypt stands ranging from 50 to 249 mg kg^{-1} of soil (average of 111 ± 23 mg kg^{-1} of soil). Variations in N mineralization rates under laboratory conditions due to season of sampling were also found by Adams and Attiwill [42], Khanna [43], Smethurst and Nambiar [38], and Theodorou and Bowen [44]. Those authors also found higher N mineralization rates during temperate summers coinciding with warm, moist soils. For Theodorou and Bowen [44,45], seasonal fluctuations in mineral N availability were related to microbial activities in the soil, which are mostly affected by temperature and soil moisture [39]. When incubating a soil sample collected during summer, a higher microbial population is also incubated, leading to a higher mineralization rate.

N_0 contents were highly correlated with N_t ($r = 0.92$; $p < 0.0001$), but less so with SOM ($r = 0.69$; $p = 0{,}0192$) and clay contents ($r = 0.83$; $p = 0{,}0015$) (Figure 3). This confirms that soil total N is a good indicator of potential N mineralization, as also found by Pottker and Tedesco [46] and Noble and Herbert [47]. However, N_0 was only weakly correlated with annual N mineralization *in situ* ($r = 0.35$), and the latter was not correlated with N_t, SOM, or clay content.

The N_0/N_t percentage varied mostly between 10 and 22% (Table 4), signifying the proportional amount of mineralizable organic N. Gonçalves *et al.* [11] found N_0/N_t percentages between 5% and 15%.

The N_t in this study accounted for 3%–5% of SOM. That ratio decreased with increased clay content (Figure 4). Therefore, there may be greater proportional N availability in soils with lower clay content. High N mineralization in sandy soils is related to better soil aeration and less clay protection of SOM. In absolute terms though, soils with higher clay content have more potential availability of N, because N_t stocks are higher.

Table 5. Solid wood volume with bark (SV) and mean annual increment (MAI) in the different treatments and ages at each site.

Site	Treatment	SV					MAI				
		$m^3\ ha^{-1}$					$m^3\ ha^{-1}year^{-1}$				
		Sandy Soils									
	Age (year)	**1.8**	**4.0**	**5.7**			**1.8**	**4.0**	**5.7**		
ALT	Control	45 b[1]	192 a	285 a			25 b	48 a	50 a		
	Usual dose	55 a	198 a	285 a			31 a	50 a	50 a		
	Increased dose	55 a	198 a	275 a			30 a	49 a	48 a		
		2.0	**3.0**	**5.5**			**2.0**	**3.0**	**5.5**		
ANG	Control	96 a	175 a	352 a			48 a	58 a	64 a		
	Usual dose	109 a	181 a	351 a			55 a	60 a	64 a		
	Increased dose	108 a	183 a	356 a			54 a	61 a	65 a		
		2.0	**3.0**	**4.0**	**5.0**		**2.0**	**3.0**	**4.0**	**5.0**	
BOT	Control	40 a	172 a	209 a	267 a		20 a	57 a	52 a	53 a	
	Usual dose	47 a	187 a	211 a	266 a		23 a	62 a	53 a	53 a	
	Increased dose	42 a	176 a	197 a	260 a		21 a	59 a	49 a	52 a	
		Loamy soils									
	Age (year)	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**
AGU	Control	52 a	132 a	186 a	257 a	313 a	26 a	44 a	47 a	51 a	52 a
	Usual dose	52 a	133 a	190 a	274 a	323 a	26 a	44 a	47 a	55 a	54 a
	Increased dose	53 a	135 a	183 a	249 a	312 a	27 a	45 a	46 a	50 a	52 a
		1.1	**2.0**				**1.1**	**2.0**			
CB3	Control	14 a	88 b				13 a	44 b			
	Usual dose	16 a	109 a				14 a	54 a			
	Increased dose	16 a	111 a				14 a	55 a			
		2.0	**4.0**				**2.0**	**4.0**			
ITA	Control	50 b	159 a				25 b	40 b			
	Usual dose	60 a	174 a				30 a	43 a			
	Increased dose	61 a	174 a				30 a	44 a			

Table 5. *Cont.*

Site	Treatment	SV						MAI					
		m^3 ha^{-1}						m^3 $ha^{-1}year^{-1}$					
		Clayey Soils											
	Age (year)	**2.0**		**4.0**		**9.0**		**2.0**		**4.0**		**9.0**	
CB1	Control	28	b	168	b	452	a	14	b	42	b	50	a
	Usual dose	33	ab	184	ab	460	a	17	ab	46	a	51	a
	Increased dose	38	a	187	a	455	a	19	a	47	a	51	a
		1.0		**2.0**		**4.7**		**1.0**		**2.0**		**4.7**	
CB2	Control	10	a	80	b	330	a	10	a	40	b	70	a
	Usual dose	11	a	92	a	332	a	11	a	46	a	71	a
	Increased dose	12	a	96	a	333	a	12	a	48	a	71	a
		1.5		**2.5**				**1.5**		**2.5**			
SMA	Control	68	a	187	a			45	a	75	a		
	Usual dose	73	a	185	a			49	a	74	a		
	Increased dose	71	a	200	a			47	a	80	a		
		2.2		**4.0**		**11.4**		**2.2**		**4.0**		**11.4**	
PAR	Control	33	a	146	a	439	a	15	a	36	a	39	a
	Usual dose	39	a	153	a	430	a	18	a	38	a	38	a
	Increased dose	42	a	153	a	430	a	19	a	38	a	38	a
		1.2		**3.1**				**1.2**		**3.1**			
VOT	Control	21	b	177	a			18	a	57	a		
	Usual dose	25	a	194	a			21	a	62	a		
	Increased dose	23	b	184	a			19	a	59	a		

[1] Values in the same column and site followed by the same letter do not differ statistically by Tukey mean test (p = 0.05).

The C/N ratio ranged from 12 to 24 (average of 19 ± 4) (Table 4). Maquere *et al.* [48], Montero [49] and Lima *et al.* [50] found higher ratios in eucalypt stands compared to other native vegetation. This explains in part the larger recalcitrance of SOM in eucalypt stands. N release slows as N mineralization proceeds, since reserves of labile N decrease along with microbial activity. This effect is unfavorable to plant nutrition in the short-term, but beneficial for N conservation in the long-term because it decreases N losses by leaching and volatilization [37,51,52]. The C/N ratio across sites was inversely correlated with N_0 (r = 0.66, p = 0.036), confirming that the more recalcitrant SOM has lower potential N availability. Although not quantified in the current study, temporal changes in C/N ratios at each site can be expected [53], whereby these ratios decrease during the early age of a rotation along with specific rates of mineralization (*i.e.*, rates of N mineralization per unit of carbon). In sandy surface soils (0–15 cm depth) supporting *Pinus radiata* stands in southeast Australia, the specific rates of N mineralization decreased by more than 50% (from 207 to 93 g N $month^{-1}$ t^{-1} C) during the first four years after planting as C/N ratios also decreased in both the <2 mm and >2 mm soil fractions [53]. For unsieved soil, the C/N ratio decreased from 38 to 31 during this period. This slow down in specific rate of N mineralization might reflect a decrease in the labile pool of organic N and organic matter quality. Such changes can be expected in any stand prior to replenishment of this labile pool as organic matter and nutrient cycling is restored by above- and below-ground litter production later in the crop rotation. Hence, the relationship

C/N ratio and N mineralization depends on poorly understood temporal and spatial factors of SOM quality.

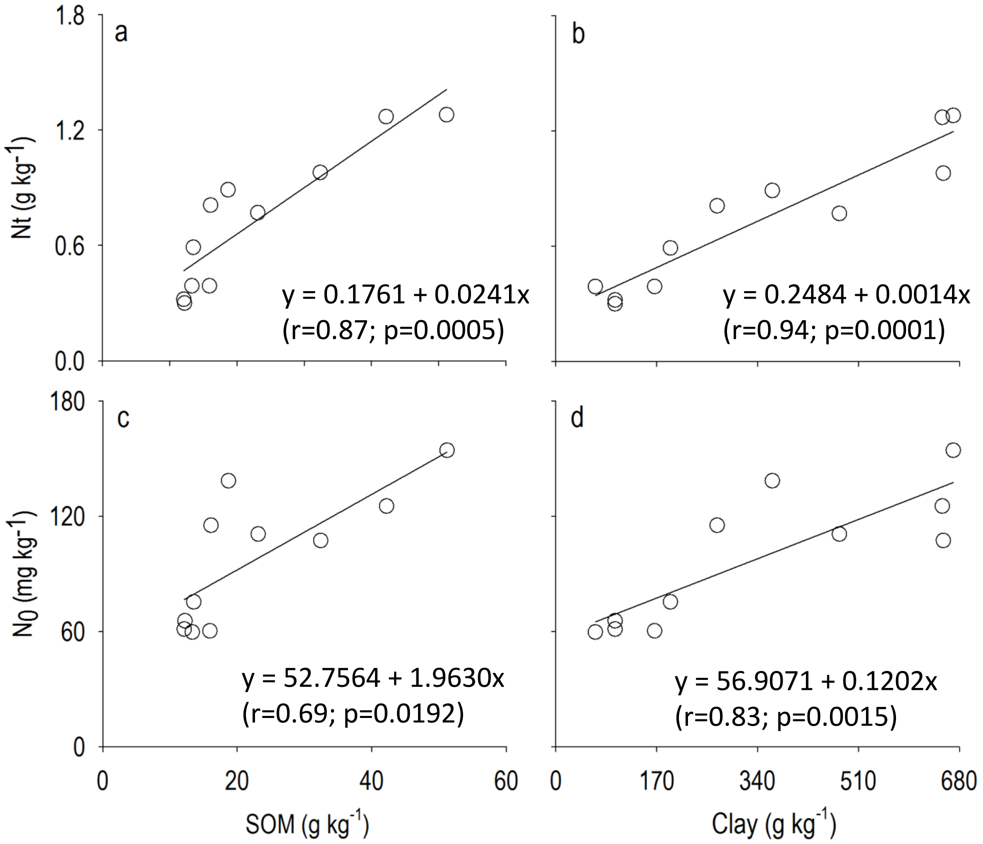

Figure 3. Correlations between soil organic matter (SOM), clay content, total N (N_t) and potentially mineralizable N (N_0).

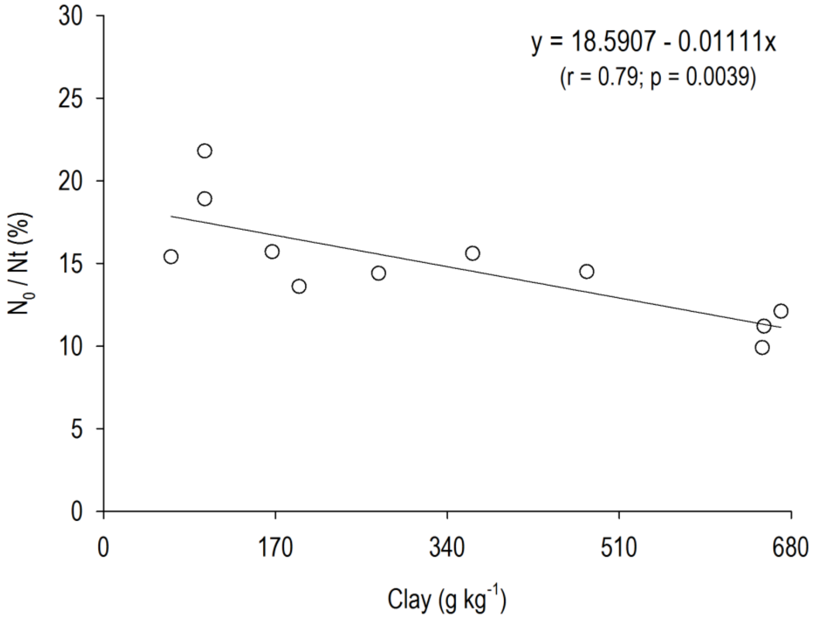

Figure 4. Correlation between clay content and N_0/N_t ratio across all sites.

4.2. Nitrogen Fertilizer Application Response

The response to N fertilizer application only occurred at an early stage of tree growth, when the canopy was in formation. For eucalypt stands in Brazil, around 70%–80% of N accumulates in aboveground components during the first two or three years of growth [1,54]. At this stage, N relative buildup is higher than biomass accumulation, due to formation of N-rich components (mainly, leaf and fine root), and N released from SOM by mineralization might not be enough to meet the N demand of trees [2]. After three years of age, competition among trees for water and light intensifies [55] and tree growth rates decline, leading to reduced N demand that is mostly supplied by N released through litter decomposition (biogeochemical cycling) and internal transfers (biochemical cycling) [1,54]. Gonçalves *et al.* [3] found a biochemical cycling of 54 kg N ha^{-1} $year^{-1}$ and biogeochemical cycling 42 kg N ha^{-1} $year^{-1}$ in *E. grandis* stands at seven years old, which are higher than the amounts required by trees (50 kg N ha^{-1} $year^{-1}$). Therefore, during early growth, N fertilizer application may accelerate tree growth rates by increasing N availability when N mineralization rates in soil and litter do not supply enough N to highly demanding trees. However, these responses disappear in subsequent years under the conditions examined here.

The mineral fertilizer requirements of any plantation depend on the nutrient demand required to reach an expected productivity, and the ability of the soil to supply this demand. When plant demand is greater than soil supply, fertilizers must be added. Thus, the criteria for N fertilizer application should involve only situations where response to N fertilizer exists, because fertilizer application aims to fill the deficit of N not released by soils. Fertilizer application practices must be linked with conservative methods of management to minimize N losses from the system, and thereby increasing sustainability [3].

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Author Contributions

Ana Paula Pulito, José Leonardo de Moraes Gonçalves, Luiz Fabiano de Moraes, José Luiz Gava, Raul Chaves and Claudio Roberto Silva designed the study and conducted of field trial. Ana Paula Pulito, José Carlos Arthur Junior, Aline Cristina Miranda and Marcos Yassuo Kamogawa were responsible for the samples collection and laboratory analysis. Ana Paula Pulito, José Carlos Arthur Junior, Clayton Alcarde Alvares and José Henrique Tertulino Rocha, were responsible for the statistical analyses, with contribution from José Leonardo de Moraes Gonçalves. Ana Paula Pulito, José Leonardo de Moraes Gonçalves, Philip J. Smethurst, Clayton Alcarde Alvares, José Henrique Tertulino Rocha and Ayeska Hübner wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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