Effect of nitrogen addition and litter removal on understory vegetation, soil mesofauna, and litter decomposition in loblolly pine plantations in subtropical Argentina

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ABSTRACT

Loblolly pine monocultures have been increasingly expanding in the Atlantic Forest of South America especially in northern Argentina. Pine plantations can modify understory vegetation and soil characteristics due to the management practices and to the dense mulch of pine needles that develop in the forest floor that could affect soil biota and ecosystem processes. Nitrogen (N) addition as expected as atmospheric deposition can also contribute to these changes. The aim of this study was to assess the effect of litter removal and low levels of N addition on understory regeneration, soil mesofauna abundance, and leaf litter decomposition. For this purpose, a completely randomized block design was used. Nitrogen addition had significant effects on understory regeneration promoting creeping herbs, graminoids and shrubs life forms affecting tree establishment and growth. Litter removal treatment showed the same pattern but only promoting the creeping herbs that could also have affected tree species. Decomposition decreased due to litter removal and was slightly increased by N addition. The addition of N decreased the abundance of mesofauna in the mulch, especially Symphypleone (a suborder of Collembola), but the abundance of the soil communities was not affected. Litter removal had a strong impact on these communities because most individuals and species of the mesofauna are present in the litter and not in the soil. This is one of the first studies analyzing the effect of low amounts of N addition and litter removal in subtropical pine plantations and contribute to understand potential impacts of increasing N deposition on biodiversity and soil processes, and to select organisms that may help as bioindicators in assessing impacts on ecological functions in productive ecosystems.

1. Introduction

Tree monocultures have been increasingly expanding in the Atlantic Forest of South America for the last three decades (Izquierdo et al., 2008; Fonseca et al., 2009). In northern Argentina, large areas of these subtropical forests have been replaced by high-yield plantations of loblolly pine (*Pinus taeda* L.). In addition to the well documented effect of tree plantations on understory vegetation and diversity (e.g. Schabenberger and Zedaker, 1999; Thomas et al., 1999; Augusto et al., 2003; Brockerhoff et al., 2003; Ramovs and Roberts, 2003; Cusack and Montagnini, 2004; Zobrist et al., 2005; Andreu et al., 2008), pine

plantations can modify soil characteristics such as pH and consequently affect soil biota and ecosystem processes and function including nutrient dynamics (Brand et al., 1986; Binkley et al., 1989; Berthrong et al., 2009). Pine plantations are largely associated with a dense mulch of pine needles on the forest floor that exudes different kinds of organic acids, tannins and phenolic compounds changing soil chemical conditions (Kanerva and Smolander, 2007; Kanerva et al., 2008). Managing practices (e.g., thinning) in tree plantations also affect soil structure by increasing compaction and decreasing water availability in the topsoil (Stogsdili et al., 1992; Zinn et al., 2002; Trentini et al., 2017).

Leaf litter plays an important role in understory regeneration and

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soil processes by changing soil microclimate conditions and nutrient availability (Facelli and Pickett, 1991a; Xiong and Nilsson, 1999; Berg and McClaugherty, 2008; Deutsch et al., 2010). In some cases, positive effects on plant establishment are due to better moisture conditions (West, 1979; Fowler, 1986) or by reducing competition with dominant species (Facelli and Pickett, 1991b). Leaf litter can also contribute to plant regeneration by preventing seed predation (Cintra, 1997). However, in coniferous plantations, leaf litter has low water-soluble constituents and high lignin content that slow down degradation processes (Berg and Staff, 1980; Coûteaux et al., 1998; Berg and McClaugherty, 2008; Kanerva et al., 2008). Pine leaf litter accumulates on the ground and acts as a barrier to plant regeneration by delaying or even impeding seed germination (Keever, 1973; Grime, 1979; Sydes and Grime, 1981; Collins and Good, 1987; Persson et al., 1987; Ellenberg, 1988; Facelli and Pickett, 1991a; Bueno and Baruch, 2011).

Besides, mulch accumulation in pine plantation can result in favorable conditions for the development of lignin degrading microorganisms such as white rot fungi (Ponge, 1991; Humphrey et al., 2000), and provide refuge and food to meso-decomposers such as springtails (Collembola) and mites (Acari) (Ponge, 1991; Sayer, 2006). Springtails and oribatid mites (suborder Oribatida) are the most dominant taxa among the soil mesofauna, both in abundance and diversity (Vlug and Borden, 1973; Ashford et al., 2013). They influence litter decomposition processes through litter comminution, which increases the surface area for bacterial and fungal activities and facilitates leaching of several compounds, but also feeding on bacteria and fungi, hence controlling their populations and dispersing them (Hansen, 1999; Wall and Moore, 1999; Adl, 2003; Barajas-Guzman and Alvarez-Sanchez 2003; Behan-Pelletier, 2003). Pine plantations can enhance mite and springtail abundances because both taxa respond positively to these typical environmental conditions, e.g. leaf-litter with high moisture contents, high C:N ratios and low soil pH (Robson et al., 2009). Litter removal was observed to affect decomposition rates directly due to the exclusion of decomposers that live in the mulch (Subke et al., 2004) and indirectly modifying soil microclimatic conditions such as temperature and water content (Ponge et al., 1993; Diaz et al., 2005; Ramakrishna et al., 2006; Sayer, 2006) both strongly correlated to litter decay (Berg and McClaugherty, 2008).

Nitrogen (N) addition can affect decomposition rates through changes in soil chemistry and soil biota, but these changes will depend on N enrichment level, litter quality and native soil community (Berg and Matzner, 1997; Knorr et al., 2005). In general, N addition promotes biological activity measured as respiration rate and microbial biomass in the forest floor (Wardle, 1992; Gallardo and Schlesinger, 1994; Berg and Matzner, 1997; Hart and Stark, 1997; Allen and Schlesinger, 2004). Native soil community and the climatic condition can also affect the direction of this effect. A recent meta-analysis performed in temperate forests suggested that N deposition inhibits the decomposition of organic matter especially in N saturated areas (Janssens et al., 2010). But effects of N addition in pine plantations in tropical and subtropical environments with N deficit litter have been less studied (Mo et al., 2006; Mo et al., 2008). In these environments, decomposition may increase when N does not reach saturating values to micro-decomposers (Mo et al., 2006). Litter quality can also affect N addition responses; Jiang et al. (2014) found that decomposition rates of pine needles (low quality) increase in response to N addition while better quality substrates decomposition was slower.

Nitrogen addition can also promote the development of primary productivity (Elser et al., 2007; Stevens et al., 2015), but with negative consequences in plant diversity due to asymmetric competition in favor of fast-growing species, which are adapted to high levels of nutrients (e.g. Vitousek et al., 1997; Bobbink et al., 1998; Stevens et al., 2004; Suding et al., 2005; Pierik et al., 2011; Borer et al., 2014, Humbert et al., 2016). Nitrogen addition can reduce tree growth due to the increase of herbaceous species coverage (Davis et al., 1999; Kraaij and Ward, 2006; Diwold et al., 2010). Besides, nutrient availability can

influence plants differently according to their ontogenetic stage (Webb and Peart, 2000), being seedlings and saplings more responsive than small trees (Alvarez-Clare et al., 2013; Fisher et al., 2013; Li et al., 2018). In general, the augment of N in soil has a positive effect on woody species growth especially under high radiation conditions (e.g., Grubb et al., 1996; Kobe, 2006). The increase of plant biomass in response to nutrient availability provides more resources for soil fauna community. N fertilization in forests can increase soil microarthropod abundance (e.g. Berch et al., 2006; Wang et al., 2016), but this has not been always observed (e.g. Maraun et al., 2001; Lindberg and Persson, 2004).

In this study, we assessed the effect of litter removal and low levels of N addition on the understory regeneration, soil mesofauna abundance and leaf litter decomposition in *Pinus taeda* L plantations. Specifically, we aimed to determine the effects on (1) plant cover and life form abundance, (2) tree seedling establishment and growth, (3) microarthropod abundance and (4) pine needle litter decomposition. We hypothesized that litter removal promotes the regeneration of woody plants and tree seedling establishment in the understory, affect negatively meso-decomposers, and decrease pine litter decomposition. Nitrogen fertilization is expected to have a positive effect on plant cover but with a detrimental effect on tree development depending on their size. Larger seedlings will have better advantages in terms of their ability to compete for the lighting resource than smaller seedlings, so they will, therefore, benefit from the addition of N. The positive effect of N addition in plant cover is expected to increase meso-decomposers abundance, and consequently pine needle decay. The study of the effect of the litter on plant regeneration, and on the characteristics and processes occurring in the undergrowth, allows us to understand the impact of needle mulch, and assess the limitations and advantages of potential management strategies. By adding low levels of nitrogen, we intended to predict likely impacts of atmospheric nitrogen deposition on some key processes and biodiversity of plants and on soil mesofauna and litter decomposition in pine plantations.

2. Methods

2.1. Experimental design

A completely randomized block design was used to test the proposed objectives. Pine plantations representing blocks were carefully selected, to have the same stand age, management and similar previous land use and being next to native forests. We only found three plantations fulfilling these conditions (n = 3). Plots were located next to native forest to avoid potential limitation to regeneration by seed arrival. In the study area the abundance and richness of seed rainfall in forest plantations is reduced to less than 20% at a distance of 100 m from the native forest (Vespa et al., 2014). Pine plantations were established in 2006 and were located between 1 km and 5 km apart from each other (see Trentini et al., 2017). Three plots of 45 × 65 m were performed in high intensity thinned stands (50%) where the treatments were randomly assigned after one year of the first thinning procedure as detailed in Trentini et al. (2017). Comparisons were made between treatments plots with N addition (N), needle litter removal (R), and a control plot (C) for both treatments in the three blocks to assess the effects separately. Because we did not intend to study potential understory management under an increment in the atmospheric deposition scenario and another treatment (i.e., litter removal combined with N addition) would decrease statistical power, we preferred no to include a factorial design. Treated and control plots were divided into six 15 × 15 m sub-plots to measure vegetation and meso-decomposers changes, and to perform litter decomposition experiments in order to avoid the hauling roads (see Trentini et al., 2017).

2.2. Nitrogen addition treatment

Fertilization was carried out with granulated urea (CH_4N_2O, 46%) in six broadcast applications during one year starting in May 2014 and until reaching $60 \, \text{kg N ha}^{-1} \, \text{year}^{-1}$. Both frequency and amount of fertilizer attempted to simulate an increase of N in the system as a result of atmospheric deposition (e.g. Lu et al., 2010, 2011). The amount of urea applied took into account that in subtropical environments the fifty percent of N could be lost by volatilization, de-nitrification, or leaching (Weier, 1994). Although, precautions were taken at application time to minimize losses. Before treatments, in March 2014, a composite sample (eighteen sub-samples per plot) were taken from the first 5 cm of soil to determine that there were no initial differences in N concentration ($F_{(1, 2)} = 2.77$; $P = 0.2$).

2.3. Litter removal treatment

Pine needle litter was removed from an internal area of $12 \times 4 \, \text{m}$ inside each sub-plot. The mulch layer ($5.6 \pm 0.82 \, \text{cm}$ deep) was completely removed. This procedure was repeated periodically in the plots to avoid mulch accumulation.

2.4. Stand structure and soil characteristics

Changes in stand structure due to N addition were determined measuring the diameter at breast height (1.3 m DBH) of all the pines higher than 10 cm of DBH in the subplot area after a year of treatment, on one block. Soil water content was estimated for the first 5 cm soil depth, in N addition, litter removal and control plots in two sampling times after treatments in October 2014 and March 2015, before and after the growing season respectively. Both samplings were done after a moderate drought of 20 days. Soil samples were oven-dried at 105 °C at constant weight. In order to determine the changes in pine mulch biomass and their water content due to N addition, three samples ($0.09 \, \text{m}^2$ each) were taken *per* treatment plot, these samples were weighed, kiln-dried at 70 °C to a constant weight. The samples were collected in June 2015. Soil and mulch water content was calculated as the difference between the weight of moist and dry samples, divided by the weight of the moist sample.

2.5. Ground coverage and understory vegetation

Changes in the understory vegetation were assessed in three quadrants of four square meters in each sub-plot. Understory ground coverage was registered in two sampling times and their change was compared between treatments and control plots. The first measure was two months before the treatments in November 2013, and the second, one year and two months after treatments in March 2015. Plant cover, branch cover, and bare soil cover (i.e. not covered by living vegetation, so include pine needle litter in N addition and control plots) were measured using the interception method (Ellenberg and Mueller-Dombois, 1974) in four points inside the quadrant ($4 \, \text{m}^2$ each). The interception method was done with a vertical stick in those points and counting all the species or branches that touch with it, up to approximately two meters high (maximum height of the undergrowth). This method is a faster sampling technique and allowed us to perform quantitative analysis to detect differences in cover with statistical models. Individuals were determined to a species when possible (in some cases only the genus could be determined) and were classified according to their life form into trees (T), shrubs (S), graminoids (G) which include Poaceae and Cyperaceae families, ferns (F), and perennial herbs (PH), creeping herbs (CH), and vines (V) that includes woody and non-woody climber species. Changes in tree seedling regeneration were estimated measuring abundance and height of all individuals by quadrant and calculating the difference between the two sampling times in treatment and control plots. Comparisons were made

separating the individuals according to two height categories: $< 0.5 \, \text{m}$ and $\geq 0.5 \, \text{m}$.

2.6. Microarthropod abundance

To assess the change in abundance of microarthropods (Collembola and Oribatida), five litter and five soil (5 cm deep) samples of about $300 \, \text{cm}^3$ were randomly taken by sub-plot in two times. Samples were taken in October 2013 and November 2014, before and after the application of the litter removal and N addition treatments, respectively. The samples were immediately taken to the laboratory to collect the fauna in Berlese-Tulgren funnels for 15 days. The microarthropods collected were fixed in 70% ethanol and observed under a stereo microscope. Springtails and oribatid mites were sorted and counted. Collembola were identified at the suborder level (Entomobryomorpha, Poduromorpha, and Symphypleona).

2.7. Decomposition

The litter-bag technique was used to estimate decomposition rates (Cuevas and Medina, 1988), which measures loss weight over time. The size of the bags used was $15 \times 15 \, \text{cm}$ and the mesh size was $2 \times 1.5 \, \text{mm}$ to allow the entry of micro and meso-decomposers (Bradford et al., 2002). In the assay 2 g of *Pinus taeda* needles were used, they were collected with traps to prevent them from reaching the ground. The collected material was not air-dried but oven-dried at 70 °C for 48 h. This was due to the high moisture content of the air in this environment which made air drying difficult, as in other studies (e.g., Padgett, 1976; Sundarapandian and Swamy, 1999; Lin and Zeng, 2018). The dry material was then placed in the litter-bags. This process allowed us to obtain initial values of dry weight while avoiding the start of the decomposition process. A total of 108 litter-bags were used, twelve for each treatment, in N addition, litter removal, and control plots. The litter-bags were placed in June 2014 and collected by duplicate six times in the months 1, 3, 6, 9, 12, and 18 after placement in the field. After collection, the remaining material inside the litter-bags was carefully separated to remove other materials (such as soil or external debris). The remaining material was dried at 70 °C for 48 h (to constant weight) and weighed.

2.8. Data analyses

The plantation stands or blocks determined the sample size in all analyses (n = 3) except in the stand structure analysis that was performed for a single block. Data were analyzed using linear mixed-effects models (LMM) where treatments (N, R, and C) were considered as fix factors and blocks (plantations stands) as a random factors in all models. LM model considers a normal distribution and an Identity link function. In some cases, a constant variance structure function (varIdent) was also added to the model to correct residual spreads. Fixed effects were assessed using likelihood ratio tests. A posteriori tests were performed by the DGC test (Di Rienzo et al., 2002). Significance levels of 5% were used but considering the low number of replicates (3) p-values lower than 0.1 were considered marginally significant. Info-Stat software was used for all analysis, as an interpreter of R (Di Rienzo et al., 2015). Treatments effects for DBH, soil water content (SWC), mulch biomass, and mulch water content (MB and MWC, respectively) were performed by comparing both treatments separately with control plots. SWC was measured twice (before and after the summer) and the analyses were performed independently. Changes in ground coverage, understory vegetation and life forms, tree seedlings abundance, and mean height were analyzed calculating the differences between the two measured times (final value minus initial value) in the same sampling quadrants for each variable (total interceptions, total life form interceptions, total number of seedling and mean quadrant height). Micro-arthropod abundance was compared between the sampling times and

layers (litter and soil) with LMM considering time, layers and their interaction as fixed factors and blocks as random factors. The change of microarthropod abundace due to the treatments was calculated by the differences between the two sampling times in soil and litter, comparing between samples taken in the same subplot. These variables were analyzed with LMM considering treatments as fixed factors and blocks as random factors. Change in decomposition rate was assessed by comparing the mass remaining. This was calculated for each litter-bag, taken from the field at each sampling time, as the difference between the final dry weight divided by the initial dry weight. The analysis was performed with LMM considering treatments, time, and treatment × time interaction as fixed factors and blocks as a random factor.

3. Results

3.1. Stand structure and soil characteristics

In fertilized plots, the average DBH of pine trees was similar among treatments ($F_{(1,65)} = 1.3$, P = 0.2). In the soil, no variations were detected in SWC between N addition and control plots, neither before nor after the growing season (SWC$_1$: $F_{(1, 25)} = 1$, P = 0.3; SWC$_2$ $F_{(1, 38)} = 0.7$, P = 0.4, respectively). Similarly, MB showed no differences between treatments ($F_{(1,14)} = 0.23$; P = 0.6). However, MWC was significantly lower in plots with N addition than in control ones ($F_{(1,14)} = 14.4$, P = 0.002). In the first measurement, SWC was lower in litter removal plots than in control plots ($F_{(1, 26)} = 6.8$, P = 0.01), but after the growing season, SWC decreased in control plots, reaching similar values to those in litter removal plots $F_{(1, 38)} = 0.02$, P = 0.8) (Table 1).

3.2. Ground coverage and understory vegetation

Plant cover was similar between N addition and control plots as well as between leaf litter removal and control plots (N: $F_{(1, 99)} = 2.4$, P = 0.1; and R: $F_{(1, 94)} = 0.2$, P = 0.6). Branches cover decreased significantly in N addition plots ($F_{(1, 94)} = 8.66$; P = 0.004) and in removal plots ($F_{(1, 85)} = 45.9$; P < 0.0001), the latter being a direct effect of the application of the treatment. There were no significant differences in bare soil cover between N addition and control plots ($F_{(1, 48)} = 1.0$; P = 0.3). On the other hand, bare soil cover increased twice after litter removal in relation to control plots ($F_{(1, 51)} = 14.4$; P < 0.001) (Fig. 1A, 1B).

3.3. Life forms

Cover change analyses for each life form showed that shrubs, graminoids and creeping herbs, increased significantly in plots with N addition (S: $F_{(1.23)} = 8.8$, P = 0.007; G: $F_{(1, 45)} = 3.9$, P = 0.05; CH: $F_{(1, 48)} = 9.7$, P = 0.003). Instead, perennial herbs (PH) and three of the most abundant life forms: trees (T), ferns (F), and vines (V), did not respond to N addition (PH: $F_{(1, 18)} = 2.9$, P = 0.1; F: $F_{(1, 50)} = 0.3$,

Table 1
Diameter at breast height (DBH, cm) of pine trees; soil water content (SWC, %), five and ten months after treatments (SWC$_1$ and SWC$_2$, respectively, %); mulch biomass (MB, kg/m^2); and mulch water content (MWC, %) one year after the application of the treatments, in N addition, litter removal and control plots. Values are means ± 1 S.E. Significant differences between treatments and control plots are indicated (**P < 0.01; *P < 0.05).

	Control	N addition	Litter removal
DBH	23.8 ± 0.6	25.1 ± 1	–
SWC$_1$	24.5 ± 1.3	24.2 ± 1.3	22.6 ± 1.4*
SWC$_2$	19.96 ± 1.2	20.4 ± 1.2	20.0 ± 1.4
MB	2.06 ± 0.13	1.98 ± 0.13	–
MWC	55.5 ± 2.59	48.3 ± 2.59**	–

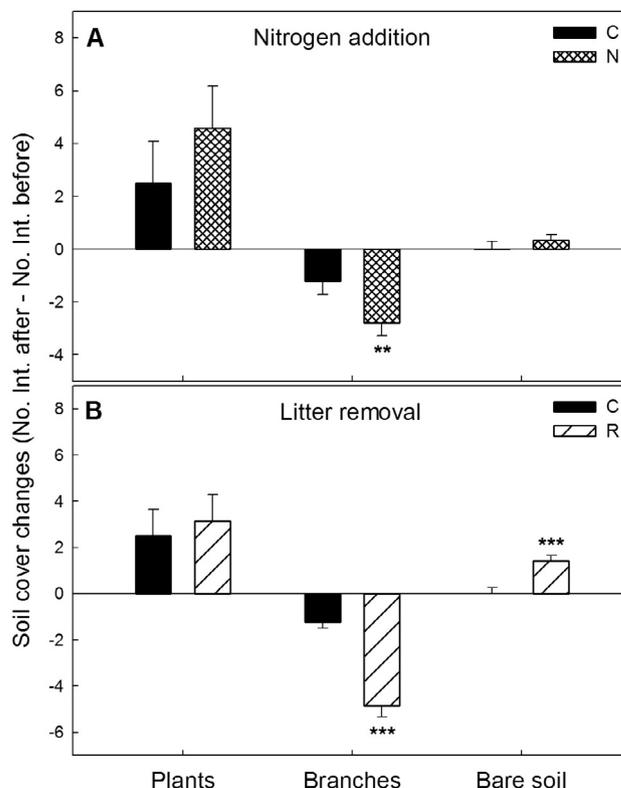**Fig. 1.** Changes in coverage at ground level measured as the difference in number of interceptions before and after N addition (▨, N) and litter removal treatments (▨, R), compared to control plots (■, C). In N addition and control plots, bare soil includes the litter layer of pine needles. Values are means ± 1 S.E. Significant differences between treatments and control plots are indicated (***P < 0.001; **P < 0.01; *P < 0.05).

P = 0.6; T: $F_{(1, 38)} = 0.5$, P = 0.5; V: $F_{(1, 64)} = 0.4$, P = 0.5) (Fig. 2A). Litter removal treatments strongly augmented creeping herbs coverage ($F_{(1, 53)} = 13.4$; P < 0.001). Shrubs showed a marginal increase after litter removal (S: $F_{(1, 31)} = 3.3$; P = 0.08). Finally, trees, graminoids, ferns, perennial herbs, and vines did not differ between litter removal and control plots (T: $F_{(1, 39)} = 0.1$, P = 0.7; G: $F_{(1,54)} = 0.2$, P = 0.7; F: $F_{(1, 57)} = 0.1$, P = 0.7; PH: $F_{(1, 22)} = 0.6$, P = 0.4; V: $F_{(1,64)} = 1.7$, P = 0.2) (Fig. 2B).

3.4. Tree seedlings regeneration

Density of understory native seedlings, shorter than 0.5 m of height, showed no differences in both N addition and litter removal treatment plots compared with control plots (N: $F_{(1, 48)} = 0.2$, P = 0.6; and R: $F_{(1, 56)} = 0.8$, P = 0.4) (Fig. 3A, 3C). Nevertheless, the abundance of tree seedlings taller than 0.5 m strongly decreased in both treatments (N: $F_{(1, 63)} = 8.8$, P = 0.004; and R: $F_{(1, 63)} = 6.3$, P = 0.01) (Fig. 3A, 3C). There were no differences in seedlings growth of individuals shorter than 0.5 m in both treatments (N: $F_{(1, 49)} = 0.36$, P = 0.5; and R: $F_{(1, 57)} = 1.7$ E^{-04}, P = 0.9) (Fig. 3B, 3D) but seedlings taller than 0.5 m showed a marked decreased in height (more than 20 cm on average per year per quadrant) with N addition, although this effect was only marginally significant ($F_{(1, 62)} = 3.6$; P = 0.06) (Fig. 3B). Litter removal did not have an effect on growth in seedlings taller than 0.5 m ($F_{(1, 64)} = 0.6$; P = 0.6) (Fig. 3D).

3.5. Microarthropod abundance

Microarthropod abundance was higher in the litter layer than in the soil in both sampling times ($F_{\text{layer x time }(1,139)} = 32.9$, P < 0.0001).
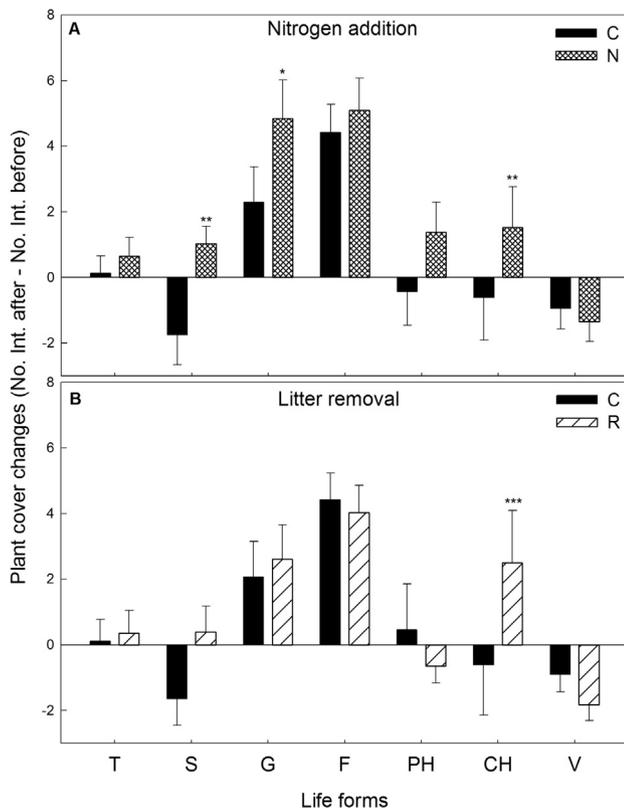
Fig. 2. Changes in plant cover, grouped by life form, measured as the difference between the number of interceptions before and after treatments in nitrogen addition (▨, N) and litter removal plots (▧, R) compared to control plots (■, C). Changes were analyzed for each life form (T: trees, S: shrubs, G: graminoids, F: ferns, PH: perennial herbs, CH: creeping herbs and V: vines). Values are means ± 1 S.E. Significant differences between treatments and control plots are indicated ($^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$).

Total number of individuals registered before and after treatments in control plots were 6862 and 2656 in the litter and 670 and 233 in soil, respectively. Generally, within the pine litter layer, abundances of the four groups of microarthropods decreased between measured times, and this reduction was higher in N addition plots compared to control plots. Decrease in Entomobryomorpha abundance due to N addition was not significant ($F_{(1, 26)} = 0.9$; $P = 0.3$), while Oribatida and Poduromorpha showed a marginally significant response to this treatment (Oribatida: $F_{(1, 26)} = 3.3$, $P = 0.08$; and Poduromorpha: $F_{(1, 26)} = 3$, $P = 0.09$). A decrease in the abundance of Symphypleona showed a significant effect of the treatment ($F_{(1,26)} = 5.4$; $P = 0.03$) (Fig. 4).

In the soil there were no differences in abundance of any of the suborders studied after the addition of N (Entomobryomorpha: $F_{(1,25)} = 1.5$, $P = 0.2$; Oribatida: $F_{(1,26)} = 0.3$, $P = 0.6$; Poduromorpha: $F_{(1,14)} = 0.01$, $P = 0.9$; Symphypleona: $F_{(1,15)} = 0.3$, $P = 0.6$) (Fig. 5A). The change in abundance of Oribatida in soil samples was lower in litter removal than in control plots ($F_{(1, 25)} = 10.3$; $P = 0.004$). Changes in Entomobryomorpha showed the same pattern but the effect was only marginally significant ($F_{(1,24)} = 2.6$; $P = 0.1$). On the other hand, Symphypleona decreased more in removal compared to control plots ($F_{(1,13)} = 5.2$; $P = 0.04$). Poduromorpha did not show a response to litter removal ($F_{(1,13)} = 0.4$; $P = 0.5$) (Fig. 5B).

3.6. Decomposition

The decomposition experiment showed that, after one year, the mass remaining was similar in N addition treatment and control plots. After 18 months, N addition plots showed lower remaining values than

control plots, indicating an acceleration in decomposition rates, although these values were marginally significant ($F_{Treat \times Time (5, 54)} = 2.03$; $P = 0.09$) (Fig. 6A). On the other hand, plots with litter removal showed higher remaining mass values over time, even one month after the start of the essay ($F_{Treat \times Time (5, 57)} = 16.4$; $P < 0.001$) (Fig. 6B).

4. Discussion

4.1. Stand structure and soil characteristics

Nitrogen addition and litter removal resulted in changes in understory plant cover, soil characteristics, litter decomposition, and mesofauna community. Nitrogen addition did not affect stand structure as expected. Other studies observed important increments in leaf production (i.e., increased total leaf area) during the first year but with higher amounts of nitrogen addition in combination with other nutrients (Valinger, 1993; Albaugh et al., 1998). Total leaf area was not addressed in our study. Although no significant structural changes were observed at the stand level in our study, there were important effects at ground level on both soil biota and undergrowth vegetation.

The amount of mulch of needle litter showed an important role in soil water retention consistent with other studies (Ginter et al., 1979; Ramakrishna et al., 2006; Deutsch et al., 2010; Rhoades et al., 2012), and supporting the positive relationship founded by Eckstein and Donath (2005) between soil water content and litter biomass. Water retention in the soil could be related to a reduced evapotranspiration as a consequence of lower temperature values found in covered soils (Rhoades et al., 2012; Xu et al., 2013). However, under water deficit conditions, soil water content in control plots (without mulch removal) decreased reaching values similar to litter removal plots (post-summer values). On the other hand, N addition can increase needle biomass by promoting pine aerial growth (Brix, 1983; Valinger, 1993). In our study, neither mulch biomass nor soil water content changed due to N addition. However, mulch water content was lower in fertilized than in control plots. This difference could be related to the increase of creeping herbs and grasses species with shallow roots.

4.2. Ground coverage and understory vegetation

We expected to find important increases of plant cover with N addition (e. g. Elser et al., 2007; Lu et al., 2010; Stevens et al., 2015). However, despite vegetation cover mean values were two-fold greater in fertilized compared to control plots, the differences were not statistically significant possibly due to the differential responses of the life forms analyzed. Some of the most abundant life forms were responsive to N addition treatments (graminoids and creeping herbs) while the others did not show differences at all (ferns and vines). Another factor that possibly limited plant development and recruitment was light availability. Nitrogen addition started one year after thinning when vegetation response was decreasing due in part to a decrease in solar radiation in the understory (Trentini et al., 2017). In this case, solar radiation could have been a factor more important than nutrient limiting plant growth (Lu et al., 2011). Consistent with this, no differences were found in bare soil due to N addition. Intercepted points without vegetation represented a very low percentage (7% and 6% in control and N addition plots at the beginning of the experiment, respectively) due to the fast understory development immediately after thinning (Trentini et al., 2017).

4.3. Life forms

Consistent with what we expected, most of the species that presented a response to nitrogen addition were fast-growing life forms with potential effects in understory simplification. Shrubs were highly responsive to N addition, which could be related to the rapid response
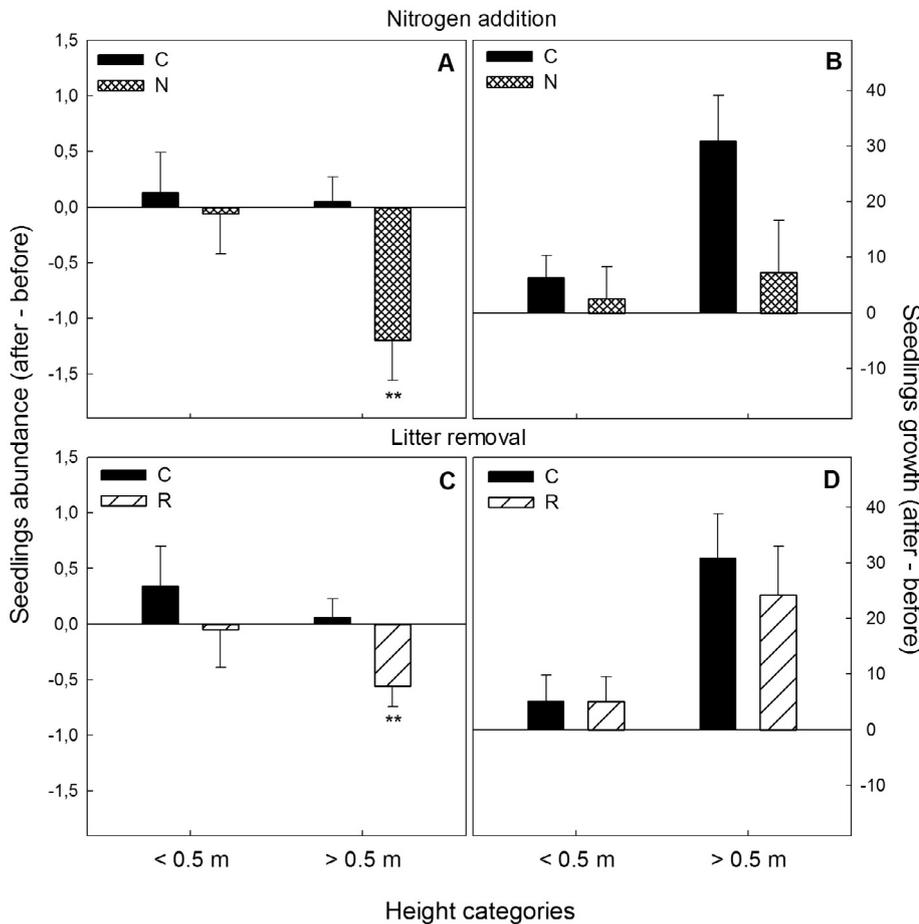
Fig. 3. Changes in seedlings abundance and height before and after N addition (N) and litter removal (R) treatments compared to control plots (C). Data were analyzed considering two height categories: lower and higher than 0.5 m. Differences were calculated in 2 × 2 m quadrants. Values are means ± 1 S.E. Asterisks indicate differences between treatments and control plots (**P < 0.01; *P < 0.05).
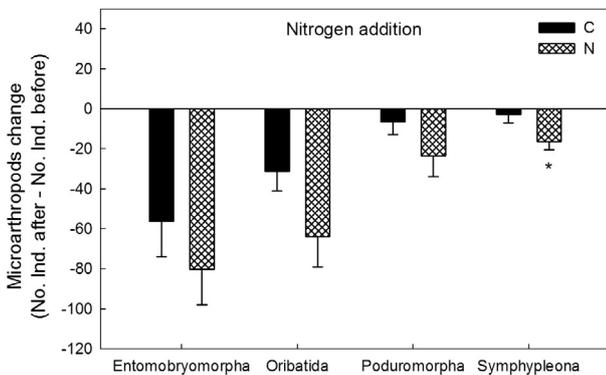

Fig. 4. Changes in microarthropods abundances in pine needle litter before and after N addition (N), and in control plots (C). Values are means ± 1 S.E. Significant differences between treatment and control plots are indicated (*P < 0.05).

that many species have after disturbances (Chazdon, 1992; Laska, 1997). For example, species of the genus *Piper* have a regrowth colonization strategy that changes the structure of the understory in a forest of Costa Rica (Gartner, 1989), and also in northern Argentina where our study was done (Campanello et al., 2007; Montti et al., 2011). Grasses, the other responsive group, are generally favored by the addition of N because of their rapid capacity to exploit available resources (Price and Morgan, 2007; Xia and Wan, 2008; Lu et al., 2010; Yang et al., 2011). Creeping herbs also had a positive response to the addition of N

treatments. It is noteworthy that clonal reproduction was a common characteristic of most of the species that constitute the three life forms mentioned before. These groups of species may have also negative consequences for the establishment of other species, particularly trees (Chapman and Chapman, 1997; Paul et al., 2004; Campanello et al., 2007, Montti et al., 2011). Some clonal species showing high plasticity in the search for resources (Keser et al., 2014) are able to modify soil chemical properties and enzymatic activity, negatively impacting the germination and growth of other species (Novoa et al., 2014). However, long term studies are needed because no differences in species richness, or in the evenness of both species and life forms, were observed over the period of time analyzed (data not shown).

Litter removal treatment did not have an effect on total plant cover contrary to what was expected in this study and observed in other studies (Deutsch et al., 2010). The increase of bare soil points could have been due to handling effects. Contrary to the positive effects expected in litter removal understory regeneration, no responses were observed in most life forms. Even though creeping herbs were the more disturbed life form, it had also the highest positive response. So, this may evidence that mulch slow down these species development possibly because their shallow roots have to penetrate a dense mulch of needles to get nutrients. Also, mulch retains water, prevents it from reaching the soil and favors evaporation after low precipitation events (Walsh and Voigt, 1977) further affecting particularly these species but also the growth rates of other life forms (Tao et al., 1987). Shrub cover increased marginally because of litter removal. It is possible that time was not enough to detect effects in life forms that have slower growth rates than creeping herbs.
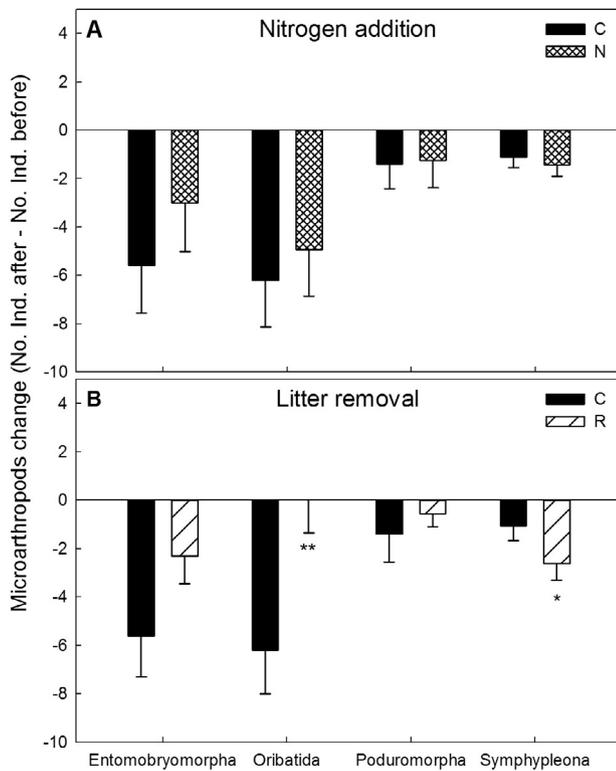
Fig. 5. Changes in the abundance of the four groups of microarthropods studied, in soil, before and after N addition (N) and litter removal treatments (R), compared to control plots (C). Values are means ± 1 S.E. Significant differences between treatments and control plots are indicated (**P < 0.01; *P < 0.05).

Fig. 6. Exponential needle litter mass loss over time in N addition (N), litter removal (R), and control plots (C). Values are means ± 1 S.E. Different letters indicate significant differences on treatment and time interaction (N addition: P = 0.09, Litter removal: P < 0.001).

4.4. Tree seedling regeneration

We expected that individuals lower than 0.5 m were affected by N addition since competition with other life forms would be strong. However, our results showed that individuals lower than 0.5 m were not affected while individuals taller than 0.5 m decreased their abundance and height considerably after treatment. One possible reason for these findings is that tree seedlings were not tall enough to avoid competition with other life forms that increased after N addition like shrubs, creeping herbs or graminoids. A second reason concerns shade tolerance. Our analysis pooled all seedlings irrespective of their shade tolerance but several studies found that shade-tolerant tree species are nutrient limited under close canopy conditions, while the light-demanding ones are not (e.g. Pasquini and Santiago, 2012; Santiago et al., 2012; Villagra et al., 2013; Chou et al., 2018). A third possible reason is related to the light environment. It is generally accepted that light is a primary limiting factor for tree growth in forest understories (Chazdon et al., 1996). As light limitation increased during the experiment, the impact of N addition on growth may have been reduced. The final reason for the absence of growth responses concerns time. In local plantations, management interventions are frequent and therefore it is not feasible to conduct a long-term fertilization experiment in the field.

Litter removal did not show the expected response of increase of seedling regeneration especially in the early stages. The lack of differences in the abundance of seedlings shorter than 0.5 m height does not support the hypothesis that mulch could be a barrier to tree recruitment. In addition, the decline of seedlings higher than 0.5 m suggests that litter removal not only does not favor the regeneration but it harms it, unlike to what was found in Tao et al. (1987). The availability of light and also a lower soil water content that might reduce desiccation
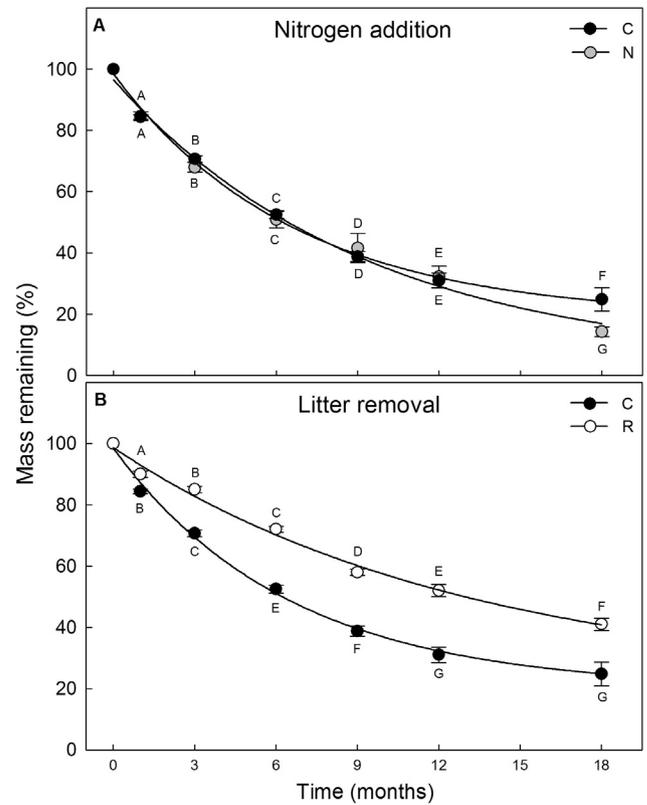
during summer may have been the main limiting factor that inhibited the expected plant responses after leaf litter removal, especially in the smaller category.

4.5. Microarthropods

The decrease of microarthropods abundances in pine litter layer of both, control and N addition plots, and also the soil of the litter removal plots, can be related to interannual variations that may be associated to local changes in climatic conditions. Furthermore, in the N addition treatment, we observed a statistically significant difference only in the litter layer. This is in agreement with other studies that show a decrease in the intensity of disturbances with depth (Vlug and Borden, 1973; Petersen et al., 2004; Coleman and Rieske, 2006). We expected that the addition of N would increase the abundance of meso-decomposers by increasing the plant biomass (plant cover) and also the microbial biomass. However, we found a reduction of Symphypleona abundance with N addition in the litter layer (from 359 individuals to 115 in N addition and from 266 to 225 in control plots) and the same pattern was observed for the other groups but differences were no statistically significant. These results show that, on a short time-scale, fertilization can have a negative effect on litter layer fauna (Lindberg and Persson, 2004). In the soil, there were no differences due to N addition. Overall, our hypothesis that litter removal would negatively impact meso-decomposers was partly supported. Symphypleona did show a negative response to this treatment, while the oribatid mites has been less affected in the treatment than in control plots. This last result partially disagrees with other studies that show a strong negative effect of litter removal on oribatid abundance in soil, by depriving the soil

communities of the protection of litter against high temperatures, drought and predators (Behan-Pelletier, 1999; Sayer, 2006; Ashford et al., 2013). In our study, heavy rainfall events prior to the second sampling time probably affected negatively microarthropod abundance, but for oribatid mites the impact was apparently lower in litter removal than in control plots. The observed changes could indicate that these decomposer taxa are sensitive to disturbances and could be used as good indicators when evaluating management. These changes should be studied further as they will allow us to deepen into the consequences of management practices.

4.6. Decomposition

An increase in the decomposition rate was expected in N addition plots according to other studies (Thirukkumaran and Parkinson, 2000; Jiang et al., 2014). However, the results were not very conclusive, just a marginally decrease was detected in mass remaining eighteen months after the experiment started. In other studies increases in pine litter decay have been found with N addition even in short periods of time (Lin et al., 2013; Mo et al., 2006) and laboratory experiments (Jiang et al., 2014). However, branch coverage was significantly lower in fertilized compared to control plots, which could be indicating an acceleration of the wood decay consistent with Zhang et al. (2016). This study attributes the increase in wood decomposition in plots with N addition to the macro-decomposers activity. Because the size of the mesh used in our study excluded macro-decomposers, and as N addition showed negative effects on meso-decomposers abundance, we could attribute this slight increase in needle litter decomposition to micro-decomposers, especially those white rot fungi present in the samples favored by the high moisture conditions of these environments.

As we expect litter removal strongly affected decomposition rate probably due to the low values of water content of the soil that may have affected soil biota communities (Benkobi et al., 1993; Geddes and Dunkerley, 1999; Prevost-Boure et al., 2011; Zhang et al., 2016). Moreover, mulch represent a structural habitat for meso and macro-fauna (McLean et al., 1996; Moseley et al., 2004), and also maintains a favorable microenvironmental conditions for microorganisms, even despite the poor quality of the litter, as was shown in a meta-analysis about litter manipulation (Xu et al., 2013). In addition, the decreased rate of decomposition may be the result of the removal of soil biota, especially of meso-decomposers, that live on mulch (McLean et al., 1996; Irmler, 2000), which were more abundant than mesofauna collected from soil samples in our study.

5. Conclusion

The development of grasses, creeping herbs, and shrubs due to nitrogen fertilization can have consequences for other species by simplifying the structure of the ground vegetation. Therefore, nitrogen deposition or even the use of fertilizers in subtropical plantations could have negative consequences for biodiversity conservation affecting other species that may provide shelter and food to animals. Similarly, but with a less pronounced effect, litter removal promoted the development of creeping herbs affecting species regeneration. Both treatments had negative effects on the regeneration and development of native tree species.

The addition of nitrogen also appeared to slightly accelerate decomposition, which may have long-term consequences on carbon storage that should be studied in more detail. In turn, the addition of nitrogen had a negative impact on the abundance of microarthropods, especially of Symphypleona in the litter layer. Litter removal negatively affected decomposition by affecting microclimatic conditions and the elimination of decomposers. Particularly, some groups of Collembola and Oribatida could be good indicators of soil disturbance and would allow assessing the sustainability of pine plantation management. In the longer term, we would expect to detect higher changes in decomposer

fauna that could potentially impact on soil processes. It is necessary to highlight the importance of the pine litter layer to maintain soil processes and biodiversity.

This is one of the first studies analyzing the effect of low amounts of nitrogen addition and litter removal in subtropical pine plantations that contribute to understanding potential impacts of increasing nitrogen deposition on biodiversity. Meso-decomposer organisms may help as bioindicators in assessing impacts on ecological functions in productive ecosystems.

References

Adl, S.M., 2003. The Ecology of Soil Decomposition. Cabi Publishing.

Albaugh, T.J., Allen, H.L., Dougherty, P.M., Kress, L.W., King, J.S., 1998. Leaf area and above-and belowground growth responses of loblolly pine to nutrient and water additions. For. Sci. 44 (2), 317–328.

Allen, A.S., Schlesinger, W.H., 2004. Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. Soil Biol. Biochem. 36 (4), 581–589.

Alvarez-Clare, S., Mack, M.C., Brooks, M., 2013. A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest. Ecology 94 (7), 1540–1551.

Andreu, M.G., Zobrist, K., Hinckley, T., 2008. Management Practices to Support Increased Biodiversity in Managed Loblolly Pine Plantations. University of Florida Extension IFAS Extension.

Ashford, O.S., Foster, W.A., Turner, B.L., Sayer, E.J., Sutcliffe, L., Tanner, E.V.J., 2013. Litter manipulation and the soil arthropod community in a lowland tropical rain-forest. Soil Biol. Biochem. 62, 5–12.

Augusto, L., Dupouey, J.L., Ranger, J., 2003. Effects of tree species on understory vegetation and environmental conditions in temperate forests. Ann. For. Sci. 60 (8), 823–831.

Barajas-Guzman, G., Alvarez-Sanchez, J., 2003. The relationships between litter fauna and rates of litter decomposition in a tropical rain forest. Appl. Soil Ecol. 24, 91–100.

Behan-Pelletier, V.M., 1999. Oribatid mite biodiversity in agroecosystems: role for bioindication. Agric. Ecosyst. Environ. 74 (1–3), 411–423.

Behan-Pelletier, V.M., 2003. Acari and Collembola biodiversity in Canadian agricultural soils. Can. J. Soil Sci. 83 (Special Issue), 279–288.

Benkobi, L., Trlica, M.J., Smith, J.L., 1993. Soil loss as affected by different combinations of surface litter and rock. J. Environ. Qual. 22 (4), 657–661.

Berch, S.M., Brockley, R.P., Battigelli, J.P., Hagerman, S., Holl, B., 2006. Impacts of repeated fertilization on components of the soil biota under a young lodgepole pine stand in the interior of British Columbia. Can. J. For. Res. 36 (6), 1415–1426.

Berg, B., Matzner, E., 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. Environ. Rev. 5 (1), 1–25.

Berg, B., Staaf, H., 1980. Decomposition rate and chemical changes of Scots pine needle litter. II. Influence of chemical composition. Ecol. Bull. 373–390.

Berg, B., McClaugherty, C., 2008. Plant Litter - Decomposition, Humus Formation, Carbon Sequestration. Springer-Verlag, Berlin.

Berthrong, S.T., Jobbagy, E.G., Jackson, R.B., 2009. A global meta-analysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. Ecol. Appl. 19 (8), 2228–2241.

Binkley, D., Valentine, D., Wells, C., Valentine, U., 1989. An empirical analysis of the factors contributing to 20-year decrease in soil pH in an old-field plantation of loblolly pine. Biogeochemistry 8 (1), 39–54.

Bobbink, R., Hornung, M., Roelofs, J.G., 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. J. Ecol. 86 (5), 717–738.

Borer, E.T., Seabloom, E.W., Gruner, D.S., Harpole, W.S., Hillebrand, H., Lind, E.M., Adler, P.B., Alberti, J., Anderson, T.M., Bakker, J.D., Biederman, J., Blumenthal, D., Brown, C.S., Brudvig, L.A., Buckley, Y.M., Cadotte, M., Chu, C., Cleland, E.E., Crawley, M.J., Daleo, P., Damschen, E.I., Davies, K.F., DeCrappeo, N.M., Du, G., Firn, J., Hautier, Y., Heckman, R.W., Hector, A., Lambers, J.H.R., Iribarne, O., Klei, J.A., Knops, J.M.H., La Pierre, K.J., Leakey, A.D.B., Li, W., MacDougall, A.S., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Mortensen, B., O'Halloran, L.R., Orrock, J.L., Pascual, J., Prober, S.M., Pyke, D.A., Risch, A.C., Schuetz, M., Smith, M.D., Stevens, C.J., Sullivan, L.L., Williams, R.J., Wragg, P.D., Wright, J.P., Yang,

L.H., 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508 (7497), 517–520.

Bradford, M.A., Tordoff, G.M., Eggers, T., Jones, T.H., Newington, J.E., 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. Oikos 99 (2), 317–323.

Brand, D.G., Kehoe, P., Connors, M., 1986. Coniferous afforestation leads to soil acidification in central Ontario. Can. J. For. Res. 16 (6), 1389–1391.

Brix, H., 1983. Effects of thinning and nitrogen fertilization on growth of Douglas-fir: relative contribution of foliage quantity and efficiency. Can. J. For. Res. 13 (1), 167–175.

Brockerhoff, E.G., Ecroyd, C.E., Leckie, A.C., Kimberley, M.O., 2003. Diversity and succession of adventive and indigenous vascular understorey plants in *Pinus radiata* plantation forests in New Zealand. For. Ecol. Manage. 185 (3), 307–326.

Bueno, A., Baruch, Z., 2011. Soil seed bank and the effect of needle litter layer on seedling emergence in a tropical pine plantation. Rev. Biol. Trop. 59 (3), 1071–1079.

Campanello, P.I., Gatti, M.G., Ares, A., Montti, L., Goldstein, G., 2007. Tree regeneration and microclimate in a liana and bamboo-dominated semideciduous Atlantic Forest. For. Ecol. Manage. 252 (1–3), 108–117.

Chapman, C.A., Chapman, L.J., 1997. Forest regeneration in logged and unlogged forests of Kibale National Park, Uganda. Biotropica 29 (4), 396–412.

Chazdon, R.L., 1992. Photosynthetic plasticity of two rain forest shrubs across natural gap transects. Oecologia 92 (4), 586–595.

Chazdon, R.L., Pearcy, R.W., Lee, D.W., Fetcher, N., 1996. Photosynthetic responses of tropcial forest plants to contrasting light environments. In: Mulkey, S.S., Chazdon, R.L., Smith, A.P. (Eds.), Tropical Forest Plant Ecophysiology. Chapman and Hall, New York, pp. 5–55.

Chou, C.B., Hedin, L.O., Pacala, S.W., 2018. Functional groups, species and light interact with nutrient limitation during tropical rainforest sapling bottleneck. J. Ecol. 106 (1), 157–167.

Cintra, R., 1997. Leaf litter effects on seed and seedling predation of the palm *Astrocaryum murumuru* and the legume tree *Dipteryx micrantha* in Amazonian forest. J. Trop. Ecol. 13 (5), 709–725.

Coleman, T.W., Rieske, L.K., 2006. Arthropod response to prescription burning at the soil-litter interface in oak-pine forests. For. Ecol. Manage. 233, 52–60.

Collins, S.L., Good, R.E., 1987. The seedling regeneration niche: habitat structure of tree seedlings in an oak-pine forest. Oikos 48 (1), 89–98.

Coûteaux, M.M., McTiernan, K.B., Berg, B., Szuberla, D., Dardenne, P., Bottner, P., 1998. Chemical composition and carbon mineralisation potential of Scots pine needles at different stages of decomposition. Soil Biol. Biochem. 30 (5), 583–595.

Cuevas, E., Medina, E., 1988. Nutrient dynamics within Amazonian forests. Oecologia 76 (2), 222–235.

Cusack, D., Montagnini, F., 2004. The role of native species plantations in recovery of understory woody diversity in degraded pasturelands of Costa Rica. For. Ecol. Manage. 188 (1–3), 1–15.

Davis, M.A., Wrage, K.J., Reich, P.B., Tjoelker, M.G., Schaeffer, T., Muermann, C., 1999. Survival, growth, and photosynthesis of tree seedlings competing with herbaceous vegetation along a water-light-nitrogen gradient. Plant Ecol. 145 (2), 341–350.

Deutsch, E.S., Bork, E.W., Willms, W.D., 2010. Soil moisture and plant growth responses to litter and defoliation impacts in Parkland grasslands. Agric. Ecosyst. Environ. 135 (1–2), 1–9.

Di Rienzo, J.A., Guzmán, A.W., Casanoves, F., 2002. A multiple-comparisons method based on the distribution of the root node distance of a binary tree. J. Agric. Biol. Environ. Stat. 7 (2), 129–142.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2015. 2015InfoStat versión,. Grupo InfoStat, FCA. Universidad Nacional de Córdoba, Argentina.

Diaz, F., Jimenez, C.C., Tejedor, M., 2005. Influence of the thickness and grain size of tephra mulch on soil water evaporation. Agric. Water Manage. 74 (1), 47–55.

Diwold, K., Dullinger, S., Dirnböck, T., 2010. Effect of nitrogen availability on forest understorey cover and its consequences for tree regeneration in the Austrian limestone Alps (Ferris, R., Peace, A. J., Humphrey, J. W., & Broome, A.). Plant Ecol. 209 (1), 11–22.

Eckstein, R.L., Donath, T.W., 2005. Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. J. Ecol. 93 (4), 807–816.

Ellenberg, D., Mueller-Dombois, D., 1974. Aims and Methods of Vegetation Ecology. Wiley, New York, NY.

Ellenberg, H., 1988. Vegetation Ecology of Central Europe. Cambridge University Press.

Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.Y., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10 (12), 1135–1142.

Facelli, J.M., Pickett, S.T., 1991a. Plant litter: its dynamics and effects on plant community structure. Botanical Rev. 57 (1), 1–32.

Facelli, J.M., Pickett, S.T., 1991b. Plant litter: light interception and effects on an old-field plant community. Ecology 72 (3), 1024–1031.

Fisher, J.B., Malhi, Y., Torres, I.C., Metcalfe, D.B., van de Weg, M.J., Meir, P., Silva-Espejo, J.E., Huasco, W.H., 2013. Nutrient limitation in rainforests and cloud forests along a 3000-m elevation gradient in the Peruvian Andes. Oecologia 172 (3), 889–902.

Fonseca, C.R., Ganade, G., Baldissera, R., Becker, C.G., Boelter, C.R., Brescovit, A.D., Campos, L.M., Fleck, T., Fonseca, V.S., Hartz, S.M., Joner, F., Käffer, M.I., Leal-Zanchet, A.M., Marcelli, M.P., Mesquita, A.S., Mondin, C.A., Paz, C.P., Petry, M.V., Piovensan, F.N., Putzke, J., Stranz, A., Vergara, M., Vieira, E.M., Joner, F., 2009. Towards an ecologically-sustainable forestry in the Atlantic Forest. Biol. Conserv. 142 (6), 1209–1219.

Fowler, N.L., 1986. Microsite requirements for germination and establishment of three grass species. Am. Midl. Nat. 131–145.

Gallardo, A., Schlesinger, W.H., 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biol. Biochem. 26 (10), 1409–1415.

Gartner, B.L., 1989. Breakage and regrowth of Piper species in rain forest understory. Biotropica 303–307.

Geddes, N., Dunkerley, D., 1999. The influence of organic litter on the erosive effects of raindrops and of gravity drops released from desert shrubs. Catena 36 (4), 303–313.

Ginter, D.L., McLeod, K.W., Sherrod, C., 1979. Water stress in longleaf pine induced by litter removal. For. Ecol. Manage. 2, 13–20.

Grime, J.P., 1979. Plant Strategies and Vegetation Processes. John Wiley, New York, NY.

Grubb, P.J., Lee, W.G., Kollmann, J., Wilson, J.B., 1996. Interaction of irradiance and soil nutrient supply on growth of seedlings of ten European tall-shrub species and *Fagus sylvatica*. J. Ecol. 827–840.

Hansen, R.A., 1999. Red oak litter promotes a microarthropod functional group that accelerates its decomposition. Plant Soil 209, 37–45.

Hart, S.C., Stark, J.M., 1997. Nitrogen limitation of the microbial biomass in an old-growth forest soil. Ecoscience 4 (1), 91–98.

Humbert, J.Y., Dwyer, J.M., Andrey, A., Arlettaz, R., 2016. Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review. Global Change Biol. 22 (1), 110–120.

Humphrey, J.W., Newton, A.C., Peace, A.J., Holden, E., 2000. The importance of conifer plantations in northern Britain as a habitat for native fungi. Biol. Conserv. 96 (2), 241–252.

Irmler, U., 2000. Changes in the fauna and its contribution to mass loss and N release during leaf litter decomposition in two deciduous forests. Pedobiologia 44 (2), 105–118.

Izquierdo, A.E., De Angelo, C.D., Aide, T.M., 2008. Thirty years of human demography and land-use change in the Atlantic Forest of Misiones, Argentina: an evaluation of the forest transition model. Ecol. Soc. 13 (2).

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.-D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. 3 (5), 315.

Jiang, X., Cao, L., Zhang, R., Yan, L., Mao, Y., Yang, Y., 2014. Effects of nitrogen addition and litter properties on litter decomposition and enzyme activities of individual fungi. Appl. Soil Ecol. 80, 108–115.

Kanerva, S., Smolander, A., 2007. Microbial activities in forest floor layers under silver birch, Norway spruce and Scots pine. Soil Biol. Biochem. 39 (7), 1459–1467.

Kanerva, S., Kitunen, V., Loponen, J., Smolander, A., 2008. Phenolic compounds and terpenes in soil organic horizon layers under silver birch, Norway spruce and Scots pine. Biol. Fertil. Soils 44 (4), 547–556.

Keever, C., 1973. Distribution of major forest species in southeastern Pennsylvania. Ecol. Monogr. 43 (3), 303–327.

Keser, L.H., Dawson, W., Song, Y.B., Yu, F.H., Fischer, M., Dong, M., van Kleunen, M., 2014. Invasive clonal plant species have a greater root-foraging plasticity than non-invasive ones. Oecologia 174 (3), 1055–1064.

Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86 (12), 3252–3257.

Kobe, R.K., 2006. Sapling growth as a function of light and landscape-level variation in soil water and foliar nitrogen in northern Michigan. Oecologia 147, 119–133.

Kraaij, T., Ward, D., 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. Plant Ecol. 186 (2), 235–246.

Laska, M.S., 1997. Structure of understory shrub assemblages in adjacent secondary and old growth tropical wet forests, Costa Rica. Biotropica 29 (1), 29–37.

Li, Y., Tian, D., Yang, H., Niu, S., 2018. Size-dependent nutrient limitation of tree growth from subtropical to cold temperate forests. Funct. Ecol. 32 (1), 95–105.

Lin, G.G., Mao, R., Zhao, L., Zeng, D.H., 2013. Litter decomposition of a pine plantation is affected by species evenness and soil nitrogen availability. Plant Soil 373, 649–657.

Lin, G., Zeng, D.H., 2018. Functional identity rather than functional diversity or species richness controls litter mixture decomposition in a subtropical forest. Plant Soil 1–15.

Lindberg, N., Persson, T., 2004. Effects of long-term nutrient fertilisation and irrigation on the microarthropod community in a boreal Norway spruce stand. For. Ecol. Manage. 188 (1–3), 125–135.

Lu, X., Mo, J., Gilliam, F.S., Yu, G., Zhang, W., Fang, Y., Huang, J., 2011. Effects of experimental nitrogen additions on plant diversity in tropical forests of contrasting disturbance regimes in southern China. Environ. Pollut. 159 (10), 2228–2235.

Lu, X., Mo, J., Gilliam, F.S., Zhou, G., Fang, Y., 2010. Effects of experimental nitrogen additions on plant diversity in an old-growth tropical forest. Glob. Change Biol. 16 (10), 2688–2700.

McLean, M.A., Kaneko, N., Parkinson, D., 1996. Does selective grazing by mites and collembola affect litter fungal community structure? Pedobiologia 40 (2), 97–105.

Mo, J., Brown, S., Xue, J., Fang, Y., Li, Z., 2006. Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China. Plant Soil 282 (1–2), 135–151.

Mo, J., Zhang, W.E.I., Zhu, W., Gundersen, P.E.R., Fang, Y., Li, D., Wang, H.U.I., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Glob. Change Biol. 14 (2), 403–412.

Montti, L., Campanello, P.I., Gatti, M.G., Blundo, C., Austin, A.T., Sala, O.E., Goldstein, G., 2011. Understory bamboo flowering provides a very narrow light window of opportunity for canopy-tree recruitment in a neotropical forest of Misiones, Argentina. For. Ecol. Manage. 262 (8), 1360–1369.

Moseley, K.R., Castleberry, S.B., Ford, W.M., 2004. Coarse woody debris and pine litter manipulation effects on movement and microhabitat use of *Ambystoma talpoideum* in a *Pinus taeda* stand. For. Ecol. Manage. 191 (1–3), 387–396.

Novoa, A., Rodríguez, R., Richardson, D., González, L., 2014. Soil quality: a key factor in understanding plant invasion? The case of *Carpobrotus edulis* (L.) NE Br. Biol. Invasions 16 (2), 429–443.

Padgett, D.E., 1976. Leaf decomposition by fungi in a tropical rainforest stream. Biotropica 166–178.

Pasquini, S.C., Santiago, L.S., 2012. Nutrients limit photosynthesis in seedlings of a lowland tropical forest tree species. Oecologia 168 (2), 311–319.

Paul, J.R., Randle, A.M., Chapman, C.A., Chapman, L.J., 2004. Arrested succession in logging gaps: is tree seedling growth and survival limiting? Afr. J. Ecol. 42 (4), 245–251.

Persson, S., Malmer, N., Wallén, B., 1987. Leaf litter fall and soil acidity during half a century of secondary succession in a temperate deciduous forest. Plant Ecol. 73 (1), 31–45.

Petersen, H., Jucevica, E., Gjelstrup, P., 2004. Long-term changes in collembolan communities in grazed and non-grazed abandoned arable fields in Denmark. Pedobiologia 48, 559–573.

Pierik, M., Van Ruijven, J., Bezemer, T.M., Geerts, R.H., Berendse, F., 2011. Recovery of plant species richness during long-term fertilization of a species-rich grassland. Ecology 92 (7), 1393–1398.

Ponge, J.F., 1991. Succession of fungi and fauna during decomposition of needles in a small area of Scots pine litter. Plant Soil 138 (1), 99–113.

Ponge, J.F., Arpin, P., Vannier, G., 1993. Collembolan response to experimental perturbations of litter supply in a temperate forest ecosystem. Eur. J. Soil Biol. 29 (3–4), 141–153.

Prevost-Boure, N.C., Maron, P.A., Ranjard, L., Nowak, V., Dufrene, E., Damesin, C., Soudani, K., Lata, J.C., 2011. Seasonal dynamics of the bacterial community in forest soils under different quantities of leaf litter. Appl. Soil Ecol. 47 (1), 14–23.

Price, J.N., Morgan, J.W., 2007. Vegetation dynamics following resource manipulations in herb-rich woodland. Plant Ecol. 188 (1), 29–37.

Ramakrishna, A., Tam, H.M., Wani, S.P., Long, T.D., 2006. Effect of mulch on soil temperature, moisture, weed infestation and yield of groundnut in northern Vietnam. Field Crops Res. 95 (2–3), 115–125.

Ramovs, B.V., Roberts, M.R., 2003. Understory vegetation and environment responses to tillage, forest harvesting, and conifer plantation development. Ecol. Appl. 13 (6), 1682–1700.

Rhoades, C.C., Battaglia, M.A., Rocca, M.E., Ryan, M.G., 2012. Short-and medium-term effects of fuel reduction mulch treatments on soil nitrogen availability in Colorado conifer forests. For. Ecol. Manage. 276, 231–238.

Robson, T.C., Baker, A.C., Murray, B.R., 2009. Differences in leaf-litter invertebrate assemblages between radiate pine plantations and neighbouring native eucalypt woodland. Aust. Ecol. 34, 368–376.

Santiago, L.S., Wright, S.J., Harms, K.E., Yavitt, J.B., Korine, C., Garcia, M.N., Turner, B.L., 2012. Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. J. Ecol. 100 (2), 309–316.

Sayer, E.J., 2006. Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. Biol. Rev. 80, 1–31.

Schabenberger, L.E., Zedaker, S.M., 1999. Relationships between loblolly pine yield and woody plant diversity in Virginia Piedmont plantations. Can. J. For. Res. 29 (7), 1065–1072.

Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J., 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303 (5665), 1876–1879.

Stevens, C.J., Lind, E.M., Hautier, Y., Harpole, W.S., Borer, E.T., Hobbie, S., Seabloom, E.W., Ladwig, L., Bakker, J.D., Chu, C., Collins, S., Davies, K.F., Firn, J., Hillebrand, H., La Pierre, K.J., MacDougall, A., Melbourne, B., McCulley, R.L., Morgan, J., Orrock, J.L., Prober, S.M., Risch, A.C., Schuetz, M., Wragg, P.D., 2015. Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. Ecology 96 (6), 1459–1465.

Stogsdili Jr, W.R., Wittwer, R.F., Hennessey, T.C., Dougherty, P.M., 1992. Water use in thinned loblolly pine plantations. For. Ecol. Manage. 50 (3–4), 233–245.

Subke, J.A., Hahn, V., Battipaglia, G., Linder, S., Buchmann, N., Cotrufo, M.F., 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139 (4), 551–559.

Sundarapandian, S.M., Swamy, P.S., 1999. Litter production and leaf-litter decomposition of selected tree species in tropical forests at Kodayar in the Western Ghats, India. For. Ecol. Manage. 123 (2–3), 231–244.

Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., Milchunas, D.G.,

Pennings, S., 2005. Functional-and abundance-based mechanisms explain diversity loss due to N fertilization. Proc. Natl. Acad. Sci. USA 102 (12), 4387–4392.

Sydes, C., Grime, J.P., 1981. Effects of tree leaf litter on herbaceous vegetation in deciduous woodland: I. Field investigations. J. Ecol. 237–248.

Tao, D.L., Xu, Z.B., Li, X., 1987. Effect of litter layer on natural regeneration of companion tree species in the Korean pine forest. Environ. Exp. Bot. 27 (1), 53–65.

Thirukkumaran, C.M., Parkinson, D., 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biol. Biochem. 32 (1), 59–66.

Thomas, S.C., Halpern, C.B., Falk, D.A., Liguori, D.A., Austin, K.A., 1999. Plant diversity in managed forests: understory responses to thinning and fertilization. Ecol. Appl. 9 (3), 864–879.

Trentini, C.P., Campanello, P.I., Villagra, M., Ritter, L., Ares, A., Goldstein, G., 2017. Thinning of loblolly pine plantations in subtropical Argentina: impact on microclimate and understory vegetation. For. Ecol. Manage. 384, 236–247.

Valinger, E., 1993. Effects of thinning and nitrogen fertilization on growth of Scots pine trees: total annual biomass increment, needle efficiency, and aboveground allocation of biomass increment. Can. J. For. Res. 23 (8), 1639–1644.

Vespa, N.I., Zurita, G., Bellocq, M.I., 2014. Functional responses to edge effects: seed dispersal in the southern Atlantic forest, Argentina. For. Ecol. Manage. 328, 310–318.

Villagra, M., Campanello, P.I., Montti, L., Goldstein, G., 2013. Removal of nutrient limitations in forest gaps enhances growth rate and resistance to cavitation in subtropical canopy tree species differing in shade tolerance. Tree Physiol. 33 (3), 285–296.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7 (3), 737–750.

Vlug, H., Borden, J.H., 1973. Soil Acari and Collembola populations affected by logging and slash burning in a coastal British Columbia coniferous forest. Environ. Entomol. 2 (6), 1016–1023.

Wall, D., Moore, J., 1999. Interactions underground, soil biodiversity mutualism and ecosystems processes. Bioscience 49, 108–117.

Walsh, R.P.D., Voigt, P.J., 1977. Vegetation litter: an underestimated variable in hydrology and geomorphology. J. Biogeogr. 253–274.

Wang, S., Chen, H.Y., Tan, Y., Fan, H., Ruan, H., 2016. Fertilizer regime impacts on abundance and diversity of soil fauna across a poplar plantation chronosequence in coastal Eastern China. Sci. Rep. 6, 20816.

Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. 67 (3), 321–358.

Webb, C.O., Peart, D.R., 2000. Habitat associations of trees and seedlings in a Bornean rain forest. J. Ecol. 88 (3), 464–478.

Weier, K.L., 1994. Nitrogen use and losses in agriculture in subtropical Australia. Fertilizer Res. 39 (3), 245–257.

West, N.E., 1979. Formation, distribution, and function of plant litter in desert ecosystems. In: In: Perry, J.A., Goodall, D.W. (Eds.), Arid Land Ecosystems. Structure, Function, and Management. IBP, vol. 16. Cambridge University Press, Cambridge, pp. 647–659.

Xia, J., Wan, S., 2008. Global response patterns of terrestrial plant species to nitrogen addition. New Phytol. 179 (2), 428–439.

Xiong, S., Nilsson, C., 1999. The effects of plant litter on vegetation: a meta-analysis. J. Ecol. 87 (6), 984–994.

Xu, S., Liu, L.L., Sayer, E.J., 2013. Variability of above-ground litter inputs alters soil physicochemical and biological processes: a meta-analysis of litterfall-manipulation experiments. Biogeosciences 10 (11), 7423–7433.

Yang, H., Li, Y., Wu, M., Zhang, Z.H.E., Li, L., Wan, S., 2011. Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. Glob. Change Biol. 17 (9), 2936–2944.

Zhang, Y., Vogel, J.G., Meek, C., Will, R., Wilson, D., West, J., 2016. Wood decomposition by microbes and macroinvertebrates, and soil CO_2 efflux vary in response to throughfall reduction and fertilization in a loblolly pine (*Pinus taeda* L.) plantation. For. Ecol. Manage. 382, 10–20.

Zinn, Y.L., Resck, D.V., da Silva, J.E., 2002. Soil organic carbon as affected by afforestation with Eucalyptus and Pinus in the Cerrado region of Brazil. For. Ecol. Manage. 166 (1–3), 285–294.

Zobrist, K.W., Hinckley, T.M., Andreu, M.G., 2005. A Literature Review of Management Practices to Support Increased Biodiversity in Intensively Managed Loblolly Pine Plantations. Rural Technology Initiative.