Soil microbial processes in a pine silvopastoral system in NW Patagonia

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Abstract The conversion of native vegetation to tree plantation (afforestation) implies a drastic change in life forms and as a consequence, changes in the microenvironmental conditions, and the quantity and quality of organic matter entering the soil. This could affect soil microbial communities and the processes catalyzed by them. In Patagonia, afforestation with exotic, fast-growing tree species was a common practice but the consequences on the ecosystem remain poorly quantified. The objective was to study the effects of pine afforestation on litter decomposition, soil organic matter, soil microbial activity and associated biogeochemical functions in a semiarid area of NW Patagonia. We hypothesized that afforestation would decrease litter decomposition rate and soil biological activity including net N mineralization, due to changes of environmental conditions and organic matter quality. We measured in situ and potential soil net N mineralization, soil microbial biomass-C, soil enzyme activities (β-glucosidase, acid phosphatase and leucin-aminopeptidase) and litter decomposition rate. We also characterized soil pH, electrical conductivity, extractable P and total C and N. Pine plantations clearly affected decomposition rates of native grass vegetation, which was 10% lower under pine canopy cover, and decreased soil microbial biomass. Acid phosphatase activity and leucin-aminopeptidase activities were also marginally reduced. On the other hand, we did not find any significant effects of pines on soil chemical properties and N transformations after 13 years of plantation. Because effects depend strongly on time, the decrease of soil microbial biomass, acid phosphatase activity and grass decomposition rate (and the trend to lower enzyme activities related to P and N) under pine cover could be an evidence of possible changes on the long-term.

Keywords Afforestation · *Pinus* sp. · Net nitrogen mineralization · Soil organic carbon · Decomposition rate

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Introduction

The implementation of sustainable practices requires knowledge of the effects on ecosystem functions and

services of land-use changes (agriculture, forestry and livestock production). At global scale, between 2000 and 2010 the area of planted forests increased by about 5 million ha per year, which mostly involved the conversion of non-forested lands to forest plantations, a process known as afforestation (FAO 2010). In arid and semiarid zones introduced tree species have also been planted as a tool for restoration of degraded lands (Nosetto et al. 2006; Grünzweig et al. 2007). In these regions domestic grazing is the main land use (Gillson and Hoffman 2007) and consequently, silvopastoral systems became a productive alternative. These systems have many potential benefits, such as higher resource use efficiency at spatial and temporal scale, and higher economic stability by providing the income of livestock production until the final tree harvest occurs (Mosquera-Losada et al. 2005; Jose et al. 2017).

However, the conversion of native vegetation to tree plantations implies a drastic modification in life forms due to changes in the microenvironmental conditions and the quantity and quality of organic matter entering the soil (Karki and Goodman 2015). Changes promoted by afforestation can affect soil microbial communities and the processes catalyzed by them. Soil enzyme assays and microbial biomass measurement have been used to study the consequences of different management practices on ecosystem functioning (Udawatta et al. 2009; Vallejo et al. 2010; Panettieri et al. 2014) since soil microorganisms are key to nutrient mineralization and carbon dynamics in soil. Several studies indicate lower microbial biomass and activities accompanying land use change from grasslands to plantations (Macdonald et al. 2009; Berthrong et al. 2009b) except for the case of phosphatase activity (Chen et al. 2008). In particular, terrestrial ecosystems are often limited by soil N availability, therefore the rate of N supply to the trees by soil microorganisms is an important function for a sustainable production. In this context, Li et al. (2014) have demonstrated that afforestation can drastically change soil N dynamics by decreasing net N mineralization rates and changing the main form of inorganic N to increased ammonium concentration.

On the other hand, afforestation contributes to anthropogenic-C sequestration. In terrestrial ecosystems, soil organic matter is the largest C pool so that the ecosystem C budget depends mainly on it. The effect of afforestation on soil C depends on many factors, such as previous land use, environmental conditions, and plantation age and management (Guo and Gifford 2002; Jandl et al. 2007). Some models predict that soil C may decrease during the first 10 years, but then C accumulation would start gradually (Paul et al. 2003). Moreover, a recent study in South America showed that arid land conversion to forest plantations increased soil organic C but the contrary occurred in humid sites (Eclesia et al. 2012). At global scale, it has been observed that *Pinus* plantations lose soil C and N during the first 20 years of growth (Berthrong et al. 2009a).

Soil C accretion is the result of the balance between C inputs and outputs and depends strongly on organic matter decomposition, which in turn is affected by microenvironmental factors as well as litter quantity and quality (i.e., chemical composition) (Swift et al. 1979). There is evidence that canopy openings modifies microenvironmental soil factors, for example, increasing soil thermal amplitude (Bahamonde et al. 2009) and soil water content (Gyenge et al. 2011). Decreases in litter decomposition under tree canopies have also been reported in different environmental conditions (Prescott et al. 2000; Bahamonde et al. 2012). However, the response of soil microbial activity (mainly measured as respiration and microbial biomass) to tree cover is variable with studies indicating no effect (Dube et al. 2013), decreased (Macdonald et al. 2009; Parfitt and Ross 2011) or increased activity under trees (Peri et al. 2015).

In Patagonia, afforestation with exotic, fast-growing tree species is a common practice. Currently, the afforested area is of about 100,000 ha increasing at a rate close to 800 ha year^{-1}, 85% of the area corresponding to *Pinus ponderosa* (Chauchard et al. 2016). Semiarid regions in Patagonia afforested with pines showed heterogeneous effects on biological and chemical soil properties (Broquen et al. 1995; Laclau 2003; Buduba 2006; Nosetto et al. 2006; Caballé et al. 2016). The objective of this work was to study the effects of land conversion from native grasslands to pine plantations on soil organic matter, microbial activity and associated biogeochemical functions in a semiarid area of northwestern Patagonia. We hypothesized that afforestation would decrease litter decomposition rate and soil biological activity

including net N mineralization, due to changes of environmental conditions and organic matter quality.

Materials and methods

Study site and experimental design

The study was carried out in Estancia Los Peucos (39º38′22″S; 71º10′04″W), Neuquen province, between November 2012 and November 2014. Soils have a sandy loam texture and are classified as Typic and Vitrandic Haploxerolls (Broquen et al. 2004). Mean annual temperature is < 10 °C and mean annual precipitation is 800 mm concentrated in winter (AIC 2015). The native plant community is classified as a grass-steppe dominated by tussock grasses (*Pappostipa speciosa* and *Festuca pallescens* with 24 and 18% basal cover, respectively) and shrubs (the most important species being *Mulinum spinosum* with 18% of basal cover); bare soil represents 18% of the total area. The main activity in the study site is Hereford cattle grazing, with a stocking rate of 10 ha per animal on average (from November to April, late spring to early autumn in the southern hemisphere). Within an area of about 2000 ha, we chose four sites (n = 4) of adjacent native grasslands (grass cover) and young (10–13 year-old at the beginning of the study) stands of *Pinus ponderosa* (pine cover), each site of about 400 m^2. At the beginning of the experiment, the average stand density was 746 trees ha^{-1}, mean tree diameter at 1.3 m (DBH) was 15.6 cm and mean total high was 6.3 m. The tree canopy cover was 43% and the aboveground radiation was 70% of the open sites. In each site, we collected soil samples along a transect at a 2 m spacing and at 0–10 cm depth. Samples were composed by four subsamples under native grass cover and eight subsamples under pine cover (between tree crowns); more subsamples were taken under pine cover trying to capture the spatial heterogeneity of pine plantation sites.

Soil properties and microclimate

Soil samples were sieved by a 2 mm-mesh and air dried to measure pH in water (1:2.5), electrical conductivity (1:5 soil:water solution) and extractable P by the ascorbic-molybdate method (Kuo 1996). For total C and N determinations soils were sieved by 0.5 mm-mesh and measured by dry combustion (Thermo Electron, FlashEA 1112). Soil bulk density was determined with a metal cylinder of 10 cm depth. Gravimetric soil water content was estimated to express the biological variables per g of dry soil. At each sampling date, soil temperature at 10 cm depth was measured with an electronic thermometer. Air temperature and precipitation were recorded at a meteorological station located in the study site. Data were registered every 15 min and hourly averaged.

Soil nitrogen dynamics

Soil net N mineralization was estimated in situ using the buried-bag method (Keeney and Nelson 1982). Briefly, two cores were taken at each sampling date with PVC tubes, 10 cm high and 5.5 cm diameter, one sample was taken for initial determination of inorganic N (t_0) and the other was placed in a polyethylene bag and incubated in the field (t_1) until the next sampling date. For potential soil N mineralization, soil samples (100 g) were incubated aerobically at 60% field capacity and 25 °C; four destructive sampling were carried out at 4, 8 and 12 weeks. In both cases, soil samples were extracted with 2 M KCl, and NH_4^+-N was determined by the indophenol-blue method and NO_3^--N by copperized Cd reduction (Keeney and Nelson 1982). In situ net N mineralization rate was calculated as inorganic N at t_1 minus inorganic N at t_0. Daily net N mineralization rates were calculated as the change in ammonium and nitrate values during the incubation period divided by the number of days of the period, and average annual rates were calculated by averaging the values over two entire years. Potential soil N mineralization was calculated as inorganic N at each incubation date minus the initial date.

Soil labile C pools and enzyme activities

Soil microbial biomass was assessed using the fumigation-extraction method (Vance et al. 1987). Potential microbial respiration was measured as CO_2 emission in 16 week-incubations at 25 °C and 60% of field capacity. Evolved CO_2 was trapped with NaOH and titrated with HCl (0.2 M) after Ba_2Cl addition. Vials containing NaOH were replaced and analyzed

at 2, 4, 7, 11 and 16 weeks; potential microbial respiration was estimated as the cumulative CO_2 evolution during the incubation period. The potential activities of three extracellular enzymes, β-glucosidase, acid phosphatase and leucin-aminopeptidase, were measured. Enzyme assays were performed using 1:10 soil:water suspensions. Aliquots of sample suspension (1 mL) were incubated at 25 °C with 1 mL of each enzyme–substrate dissolved in buffer (5 mM pNP-β-D-glucopyranoside for β-glucosidase, 5 mM pNP-phosphate for acid phosphatase, 2 mM leucine p-nitroanilide for leucine-aminopeptidase). We used buffer acetate (pH 5, 50 mM) for β-glucosidase and acid phosphatase; and buffer TRIS (pH 8, 50 mM) for leucine-aminopeptidase. A sample control (sample suspension + buffer) and a substrate control (substrate + buffer) were also incubated.

Litter decomposition

A factorial experiment design with plant cover and time as main factors was carried out. Senescent needles of pine and main grass species of the understorey were collected in November 2012. At the same moment, senescent leaves of main grass species were collected in the adjacent native grassland. Collected materials were taken to the laboratory and air dried at room temperature to constant weight. Samples of 5 g of each material were enclosed in 10 × 10 cm polyethylene gauze litterbags (2 mm mesh). Subsamples of 3 g were oven dried for 48 h at 60 °C to calculate moisture content. For decomposition of pine senescent needles, two microsites (under and between tree crowns) were used, while for decomposition of grass litter three microsites (under and between tree crowns, and adjacent native grasslands) were evaluated. For both cases, the time factor had six levels corresponding to dates of litter bags collection (55, 148, 238, 338, 518 and 728 days). In the laboratory, the material was removed from the litterbags, cleaned of external material, and weighed after drying at 60 °C for 48 h. Corrections for inorganic contaminants (mainly soil particles) were made after determining loss on ignition of all samples (4 h, 500 °C). Remaining organic matter (ROM) was estimated as $(OM\ t_i - OM\ t_x)/OM\ t_i \times 100$, where $OM\ t_i$ is the initial organic matter (in grams) and $OM\ t_x$ is the organic matter (in grams) at each sampling date. Percentage of decomposition was calculated as

$100 - ROM$. Also, samples of pine needles and grasses were analyzed for determination of initial total C and total N concentrations. Carbon was determined by calcination in muffle furnace at 450 °C and N was determined by semi-micro Kjeldahl.

Statistical analysis

Mean differences between grass cover and pine cover were calculated with the independent two-sample Students t test for soil properties, microbial biomass and enzyme activities. Soil microclimatic variables, nitrate, ammonia, nitrification, mineralization (in situ and potential), ammonification and decomposition rates were analyzed with repeated measures ANOVA with microsite as an inter-subject factor and each sampling date as an intra-subject factor. Differences between means were evaluated with the Tukey test. Potential respiration violated sphericity assumption, even after transformation, so comparisons with independent two-sample Students t-test at each date were performed. Pearson correlations among average of the three dates of biological variables (microbial biomass and enzyme activities), initial measurements of chemical variables (pH, electrical conductivity, P-Olsen, total C and N) and percentage of decomposition at the final date were conducted (n = 8). Statistical analyses were carried out in Statistica (StatSoft 1984–2003). All results are expressed as mean ± SE and null hypothesis was rejected at $P < 0.05$.

Results

Soil properties and microclimate

Soil microclimatic variables varied across seasons, but there were no significant differences in soil temperature and moisture between grass and pine cover (Fig. 1). Similarly, soil pH, electrical conductivity, P-Olsen, and total C and N did not change significantly after the grassland was converted to pine plantation (Table 1), although C and nutrients tended to be consistently lower under pines.

Fig. 1 Atmospheric temperature (squares) and precipitations (bars) during September 2012 to November 2014 (**a**). Soil temperature and moisture at 10 cm depth during November 2012 to April 2014 under grass and pine cover (**b**). Error bars represent standard error. Results for repeated measures ANOVA were indicated
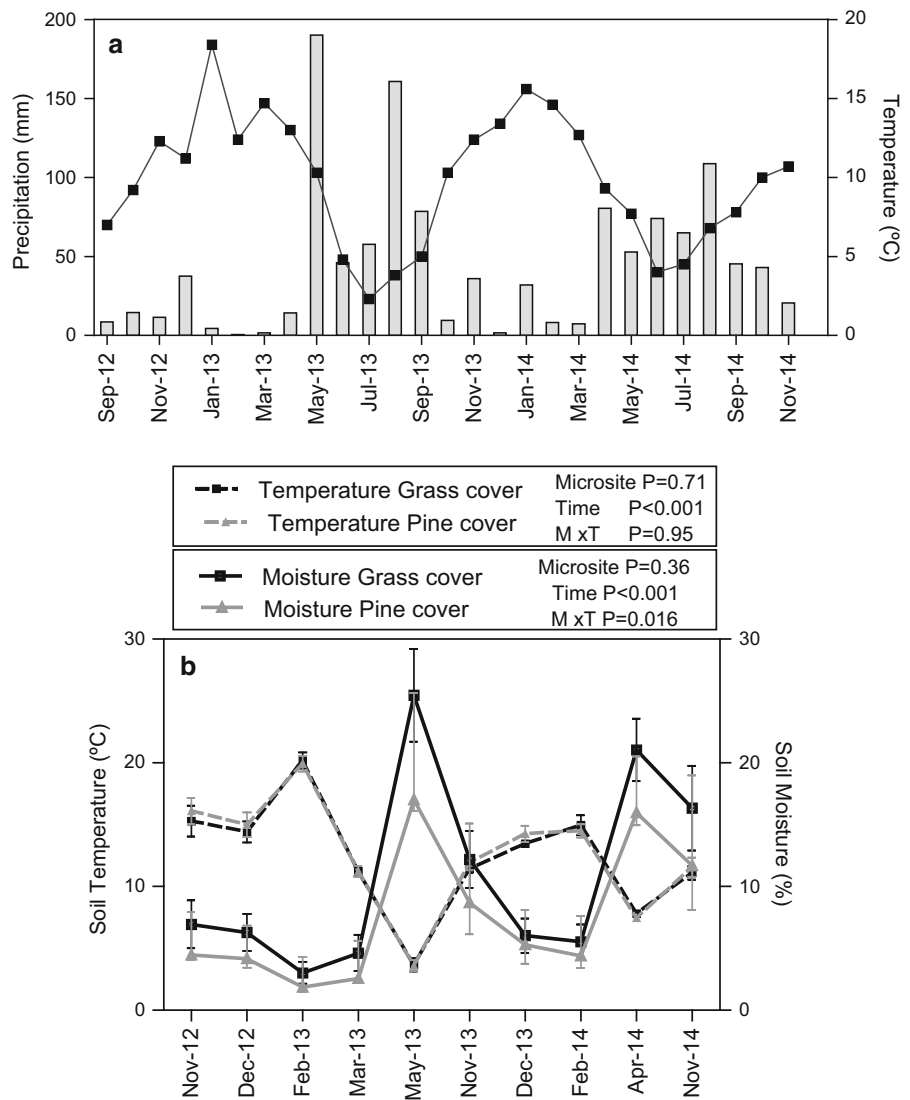

**Table 1** Soil chemical and physical properties under grass and pine cover

	Grass cover	Pine cover
pH	6.3 (0.09) a	6.5 (0.06) a
Electrical conductivity ($\mu S \ cm^{-1}$)	26.2 (2.82) a	29.1 (3.54) a
Total C (g kg^{-1})	25.2 (6.21) a	16.1 (3.12) a
Total N (g kg^{-1})	1.7 (0.09) a	1.0 (0.04) a
C:N	15	16
P-Olsen (mg kg^{-1})	20.6 (6.98) a	14.4 (1.76) a
Bulk density (g cm^{-3})	0.86 (0.078) a	0.99 (0.040) a

The letters a indicate significant differences

Soil nitrogen dynamics

Inorganic mineral N in soils clearly varied over time associated with seasonal changes, however, no detectable differences were observed for nitrate and ammonium concentrations between grassland and pine plantation (Fig. 2). Similarly, annual average of soil nitrate and ammonium did not differ between grass and pine cover, and there was not a predominant form of inorganic N, NH_4^+-N and NO_3^--N ranged from 5.4 to 0.4 and 5.0 to 0.5 mg kg^{-1}, respectively, throughout the 2-year study period (Fig. 2). Nitrogen transformation rates did not also change with tree cover, but both net N mineralization and nitrification

Fig. 2 Soil inorganic N under grass and pine cover: nitrates concentration (**a**) and annual average of nitrate (**b**), ammonium concentration (**c**) and annual average of ammonium (**d**). Error bars represent standard error. Results for repeated measures ANOVA were indicated. For annual averages independent two-sample Students *t*-test was performed
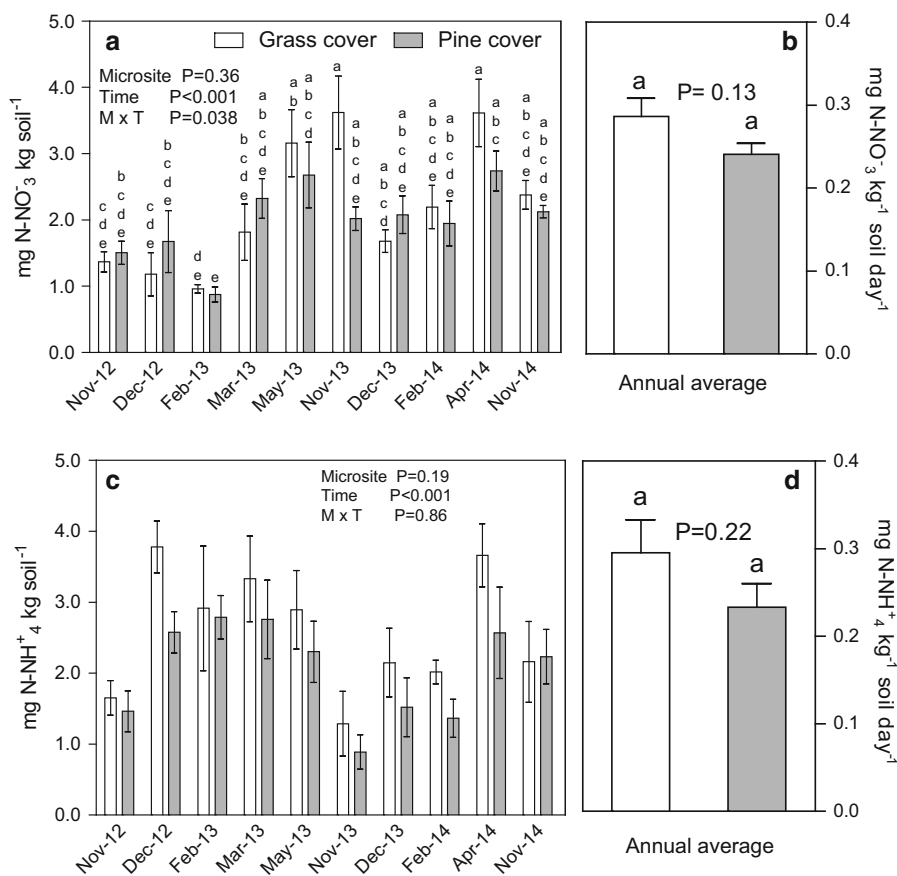
rates varied seasonally being significantly higher in late spring (Nov–Dec) of year 2013 (Fig. 3); annual net nitrification was higher than ammonification. Annual net N mineralization and nitrification under grass cover tended to be lower than under pine cover. On the contrary, potential N mineralization tended to be higher in soils from grass cover (Fig. 4a).

Soil respiration, enzyme activities and microbial biomass

Soil microbial biomass was significantly higher under grasses than under pine cover in November 2013 and 2014 (Fig. 5a). Soil potential respiration did not differ between pine and grass cover, although tended to be higher under grasses (Fig. 4b). Soil enzyme activity involved in C degradation was unaffected by the change of plant cover (Fig. 5b), while acid phosphatase and leucin-aminopeptidase activities were marginally lower under pine cover in November 2012
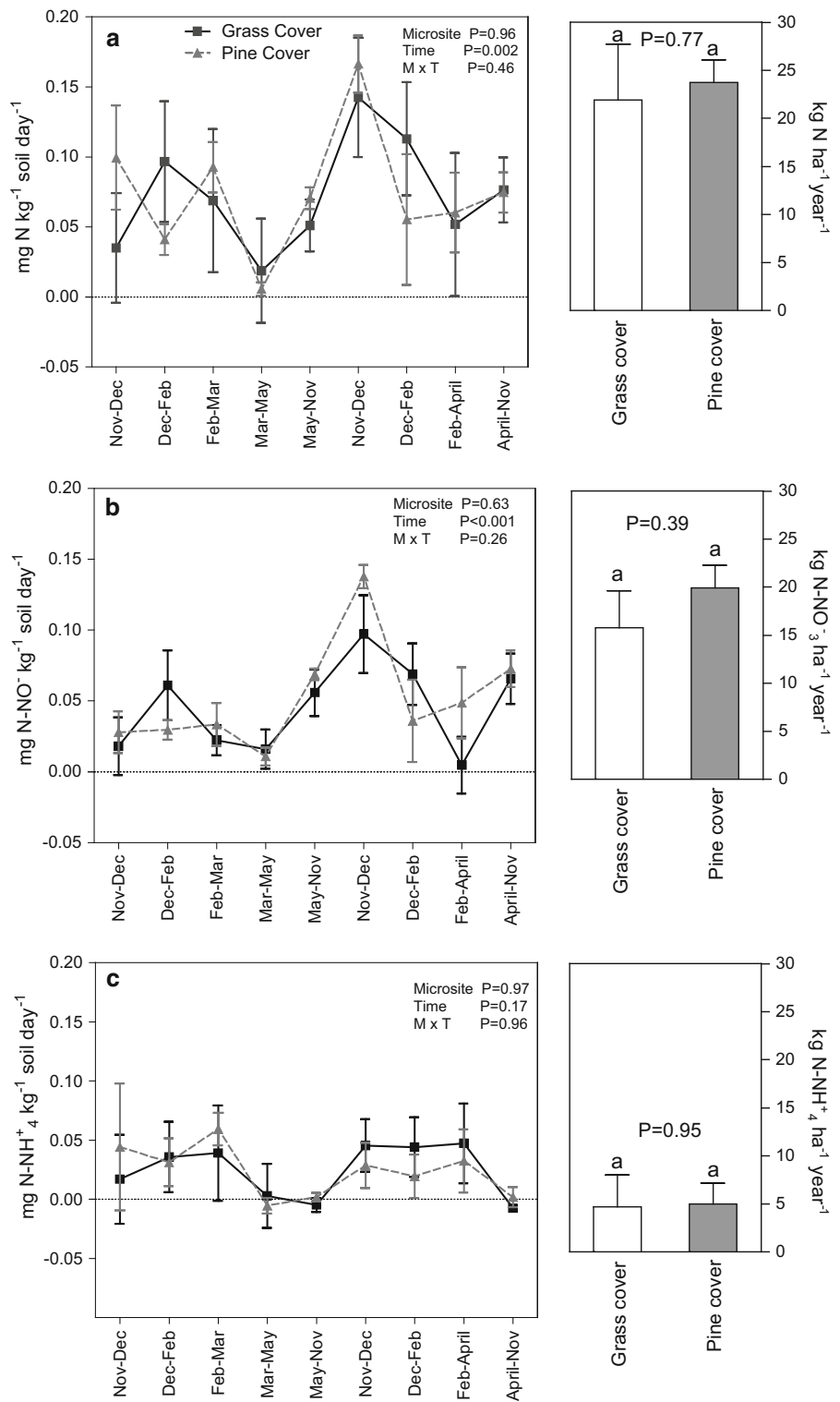
and 2014 (Fig. 5c), and November 2014 (Fig. 5d), respectively.

Litter organic matter decomposition

Carbon initial concentration did not vary significantly between litter types (grasses and pine needles) with average values of 47%. On the contrary, N concentrations were significantly different ($P = 0.018$) with values of 1.12% and 0.41% for pine needles and grasses, respectively. Therefore, C:N ratio was significantly higher ($P = 0.003$) in grasses ($= 109$) than in pine needles ($= 43$).

Grass leaf decomposition was similar between microsites during the first year, but it showed significant differences during the second year, being higher under grass cover (Fig. 6a). At the end of the evaluation period, the percentages of grass leaf decomposition were 14, 11 and 25% under pine crowns, between crowns and under grass cover, respectively. On the other hand, no differences were observed for pine

Fig. 3 Nitrogen
transformation in soil:
in situ net mineralization
(**a**); in situ net nitrification
(**b**) and in situ net
amonification (**c**) under
grass and pine cover. Error
bars represent standard
error. Results for repeated
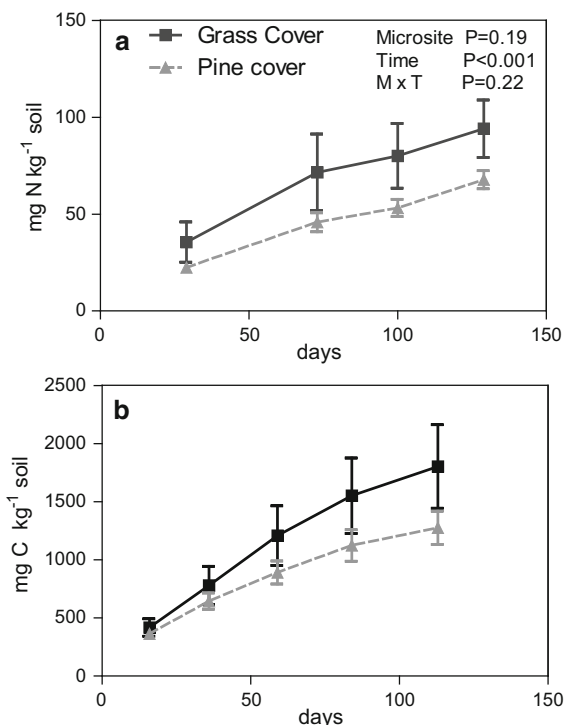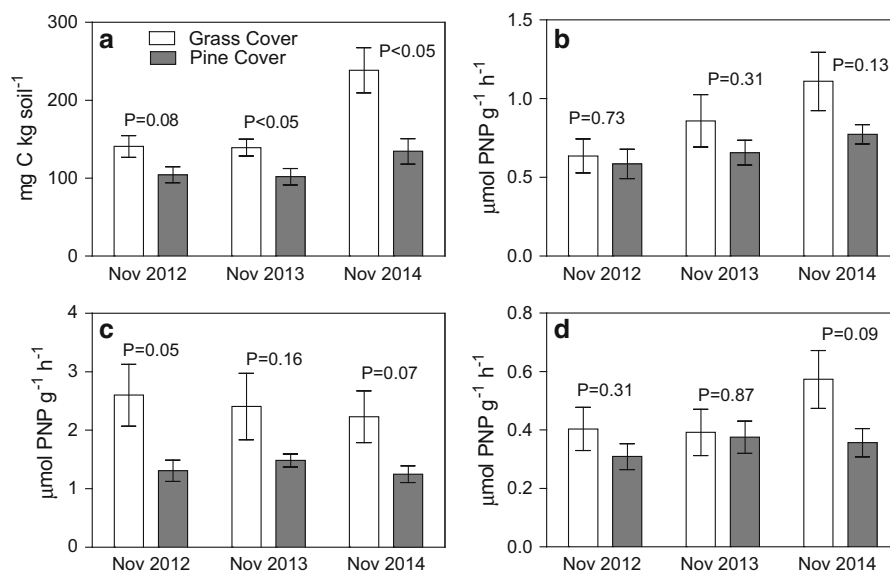measures ANOVA were
indicated

needle decomposition between microsites (under and between crowns), averaging 26% of decomposition (Fig. 6b). In both microsites of pine cover, pine needle decomposition was higher than grass decomposition at the end of the evaluated period.

Discussion

Land conversion from native grasslands to pine plantations clearly affected decomposition rates of grasses: under pine cover the percentage of grass leaf decomposition decreased by 10%. Also, soil microbial biomass and, less markedly, acid phosphatase and leucin-aminopeptidase activities decreased under pine cover. However, although this drastic land-use change could affect soil N transformations, we did not find any significant effects in this 10–13 year-old pine stands.

Changes in soil properties and soil organic C after afforestation depends on the complex interaction between prior land use, climate and tree species planted (Berthrong et al. 2012; Eclesia et al. 2012; Li et al. 2012). In agreement with Broquen et al. (1995), who worked in quite similar sites than ours in semiarid Patagonia, pH did not change under pine cover at 0–10 cm soil depth. However, a decrease in soil pH with pine and conifer plantations has been often reported (Berthrong et al. 2009a, 2012; Parfitt
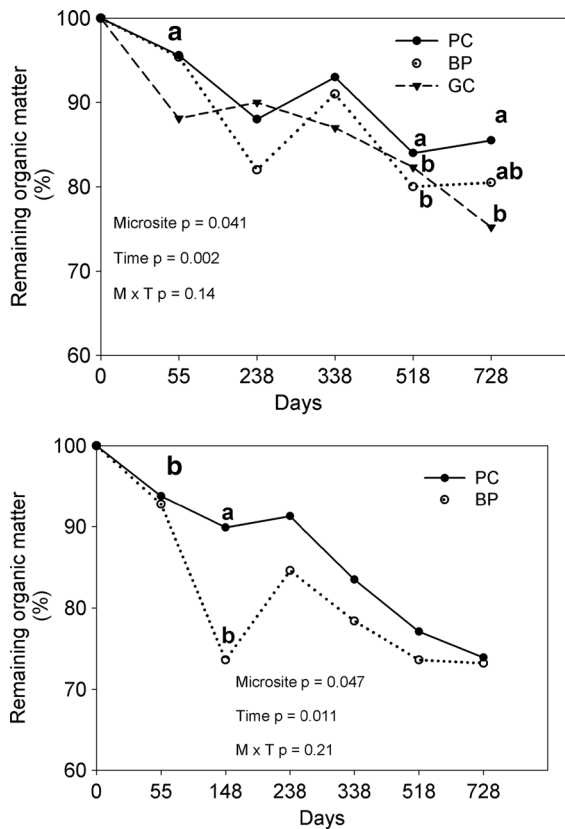
Fig. 4 Potential N mineralization (**a**) and potential respiration (**b**) in soil sampled under grass and pine cover. Error bars represent standard error. P-values for repeated measures ANOVA were indicated for potential mineralization. For potential respiration there are no significant differences between grass and pine cover (independent two-sample Students *t*-test) at each sampling date (P > 0.1)

Fig. 5 Microbial biomass and soil enzyme activities under grass and pine cover at three sampling dates. Microbial biomass-C (**a**), β-glucosidase activity (**b**), acid phosphatase activity (**c**) and leucine-aminopeptidase activity (**d**). Error bars represent standard error, the results of the independent two-sample Students *t*-test are indicated

Fig. 6 Variation of remaining organic matter (as a percentage of the initial weight) over time of grass leaf (**a**) and pine needle (**b**). *PC* pine cover, under crown, *BP* pine cover, between pines, *GC* grass cover. Different letters in the same date indicate significant differences ($P < 0.05$)

and Ross 2011). Our results indicated that soil total C did not significantly change after 13 years of plantation. Although the lack of significance is likely due to the high variability of this property, studies at global level have shown that soil organic C increases with time after afforestation (Berthrong et al. 2012), and that before 30 years of plantation soil C is depleted or changes are negligible (Li et al. 2012). The latter authors also found that soil C (considering organic and mineral layers) did not change under softwoods, such as pine, but increased under hardwoods. In addition, another meta-analysis showed no impact of afforestation of previous grasslands on C accumulation at shallow (37 cm in average) soil depths (Laganière et al. 2010). Taken together, it is not surprising the lack of changes we found on soil C after only 13 years of pine planting in native grasslands. Moreover, the semiarid conditions

prevailing in the site would contribute to retard the processes of decomposition and soil C incorporation.

Soil total N depends on the degradation of organic matter so, similarly to soil total C, it depends on the age of plantation. In our study, soil N remained unchanged after 13 years of plantation; similarly, Li et al. (2012) reported that soil total N was not affected after 50 years of vegetation replacement. On the other hand, the land-use change from native vegetation to tree plantations implies changes in biotic and abiotic conditions that could affect N dynamics; thus, some authors found a decrease in soil N mineralization in plantation compared to grasslands (Peichl et al. 2012; Li et al. 2014). Zeller et al. (2000) suggested that the increase of soil C:N ratio would be the cause of lower N mineralization in afforested lands. However, in our study the C:N ratio remained unaffected, and the main change in N transformations were due to seasonal variations that resulted in a peak of mineralization rate during late spring (November–December), when biological activity was not limited neither by soil moisture as in summer nor by low temperatures (Peri et al. 2015). Moreover, potential N mineralization rates under laboratory conditions (0.7 and 0.5 mg kg^{-1} day^{-1} under grass and under pine cover, respectively) were 17-fold higher than in situ mineralization (0.04 and 0.03 mg kg^{-1} day^{-1} under grass and pine cover, respectively), indicating that environmental conditions exert a strong control on N dynamics in the field, as also found by other authors in Patagonia (Mazzarino et al. 1998; Bahamonde et al. 2013). Finally, ammonium and nitrate pools were quite balanced, contributing to reduce the risk of N losses from the ecosystem. It would be possible that a higher number of replicates would be required to detect differences in highly variable chemical properties (total C and N, and nutrients). The trend to decreased organic matter (soil total C and N at 0–10 cm depth) found in the pine cover could be due to the reduced root density at the surface because of grass cover reduction. However, as other authors reported (Mazzarino et al. 1998; Ndiaye et al. 2000; Nunes et al. 2012), we found significant changes in some biological properties that were less variable and responded more rapidly to management practices.

Soil enzyme activities are clearly influenced by vegetation type and management practices (Acosta-Martínez et al. 2008; Bastida et al. 2008). For

example, it has been found that introduced pine species reduce soil enzyme activities, possible associated to reductions in soil organic matter and nutrients (Rutigliano et al. 2004; Hess and Austin 2017). Our results showed a marginal decrease of acid phosphatase and leucin-aminopeptidase activities under pine cover, but enzyme activities involved in C degradation were unaffected. In particular, the phosphatase enzyme activity decrease could be related to the presence ectomycorrhizal fungi in pines that facilitate mineral P hydrolysis (Plassard and Dell 2010).

Grass decomposition rates found in this study were lower than those reported by Bahamonde et al. (2012) in *Nothofagus antarctica* forests under silvopastoral use in southern Patagonia. On the other hand, the decomposition rates of pine needles at the end of the evaluated period were similar to that reported by Dube et al. (2013) for *P. ponderosa* plantations in Chilean Patagonia. Chemical composition of both litter types (grass leaves and pine needles) could be the cause of differences in decomposition rates. The dominant perennial grasses are characterized by high N resorption during senescence that results in low N concentrations in senescent leaves (Carrera et al. 2003; Bertiller et al. 2006). Grasses have, then, a higher C:N ratio than pine needles that could explain lower decomposition and N release (Swift et al. 1979; Seneviratne 2000; Cornwell et al. 2008). The decrease in decomposition rate found under tree cover has been also reported in other Patagonian ecosystems, and related to less incident radiation (Bahamonde et al. 2012; Araujo and Austin 2015). Solar radiation may have a direct action on organic matter decomposition through photodegradation (Austin and Vivanco 2006) or indirectly increasing microbial enzyme accessibility to plant litter carbohydrates (Austin et al. 2016).

Soil microorganisms are responsible of organic matter decomposition and mineralization, represent a reservoir of nutrients, and play a key role in soil stabilization by formation of stable aggregates (Rilling et al. 2003). As in other young pine afforestations (Macdonald et al. 2009; Parfitt and Ross 2011), our results showed that soil microbial biomass decreased under pine cover, and was positively correlated with soil total C (r = 0.91, P < 0.05) and soil total N (r = 0.91, P < 0.05). Moreover, it has been proposed that the cell wall envelopes of bacteria and fungi significantly contribute to soil organic matter formation (Miltner et al. 2012), so that lower microbial biomass also implies limited soil C accretion in the long-term. On the other hand, the positively correlation between microbial biomass and enzyme activities with soil total C and N, highlights the potential use of some biological variables as early indicators of disturbance.

Our results showed that after 13 years of pine plantation in a native grassland, there were no significant changes in N mineralization and availability, but decomposition rates of grasses and soil microbial biomass were decreased and a clear trend to lower activities of some enzymes were observed, which could be suggesting a decrease in C and N soil stocks in the long-term. The lack of significant effects of afforestation on soil chemical and some biological properties could be explained by a high spatial heterogeneity, but also by the identity of the planted species, prior land use, the soil´s capacity to protect and accumulate organic matter and the time elapsed since the disturbance. Some studies found that coarse textured soils have a low capacity to accumulate soil organic matter after afforestation, and that no change of C stocks occurs when clay contents are < 33% (Laganière et al. 2010). Our soils are xeric Mollisols with slightly weathered pyroclastic materials, so that physical and chemical protection of organic matter would be expected (Broquen et al. 2004). Besides, pre-planting disturbance that enhances C losses (Qiu et al. 2012) was minimal since previous vegetation was not removed from the site. Then, it might be expected that the expression of pine afforestation effects would require more time. Our results depict the importance of long-term studies to assess the effects of afforestation on soil processes, and the value of soil microbial biomass as an early indicator of changes.

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