



Spatial pattern and scale of soil N and P fractions under the influence of a leguminous shrub in a *Pinus canariensis* forest

A. Rodríguez ^{a,*}, J. Durán ^a, J.M. Fernández-Palacios ^b, A. Gallardo ^a

^a Department of Physics, Chemical and Natural Systems, Pablo de Olavide University, Seville 41013, Spain

^b Department of Parasitology, Ecology and Genetics, La Laguna University, La Laguna 38207, Spain

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ABSTRACT

Nitrogen-fixing plants alter the chemical properties of the soil beneath plant canopies, particularly by concentrating nitrogen-rich organic matter. We hypothesize that the presence of a legume canopy inside a plot will more greatly influence the spatial structure of soil nitrogen (N) than phosphorus (P). We also investigated whether the effects of legume individuals on the soil properties beneath their canopies might be mediated by soil texture and water availability. Thus, we expected that the local effect of a legume canopy would be more conspicuous in nutrient-poor sandy soils than in nutrient-rich loamy soils. Moreover, the spatial pattern should differ during the wet (winter) and dry seasons (summer) because the microbial processes driving nutrient cycling are sensitive to water availability. To test these hypotheses, square plots (4 m × 4 m or 3 m × 3 m) were placed around isolated mature individuals of *Adenocarpus viscosus* in two pine forest stands of the Canary Islands (Spain) with contrasting soil textures (loamy and sandy soil). The spatial pattern and scale of microbial biomass-N (MB-N), dissolved organic-N (DON), and inorganic-N and -P fractions (NH₄-N, NO₃-N and PO₄-P) were analyzed with geostatistical methods for two sampling dates (summer and winter). Soil variables with spatial structure demonstrated a greater spatial dependence in the loamy than sandy soil, with the exception of MB-N during summer. Except for NH₄-N and NO₃-N in winter plots, the spatial range was also lower in the sandy than the loamy soil. The legume canopy only had a clear effect on the spatial pattern of winter NH₄-N, NO₃-N, and DON in the sandy soil; no dependence was observed for PO₄-P on the legume canopy in both soil types. Our results suggest that the presence of *A. viscosus* individuals may be an important source of spatial heterogeneity in the N content of the soil in these forests. However, soil texture and water content modulated the magnitude of the legume canopy effect on the spatial distribution of these N forms beneath canopies.

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1. Introduction

Plants are important in the regulation of soil nutrient availability and distribution (Gross et al., 1995; Augusto et al., 2002; Okin et al., 2008). These photosynthetic organisms alter the physical, chemical, and biological properties of the soil beneath plant canopies, particularly by concentrating biomass and organic matter (Jackson and Caldwell, 1993a, b; Schlesinger et al., 1996; Gallardo et al., 2000). While the local plant–soil interaction has a greater effect on soil than other factors, such as topography or soil texture, there may be a mosaic pattern in soil properties formed by the influence circles of single plants (Zinke, 1962; Saetre, 1999; Gallardo, 2003a). In turn, spatial patterns of soil nutrients influence the individual functioning of plants (Antonovics et al., 1987; Gallardo et al., 2006; Quilchano et al., 2008), and ultimately the structure and function of plant communities and ecosystems (Tilman,

1988; Hutchings et al., 2003; Maestre and Reynolds, 2007). Thus, the spatial relationship between plants and soil is clearly bi-directional (Ettema and Wardle, 2002; Covelo et al., 2008; Zhou et al., 2008). These spatial patterns and scales also vary temporally, and even within a single growing season. Therefore, plants should acquire soil resources that vary in time and space, but also nutrients that exhibit temporal changes in spatial pattern and scale (Ryel et al., 1996; Cain et al., 1999).

Both nitrogen (N) and phosphorus (P) are the essential nutrients that most frequently limit primary production in terrestrial ecosystems (Vitousek and Howarth, 1991). A number of processes tend to reduce the biological availability of N in terrestrial ecosystems, such as the strong link between organic-N and recalcitrant-C compounds, as well as the mobility of N out of ecosystems, especially through leaching and denitrification (Vitousek et al., 2002). Nitrogen-fixing plants can increase soil N content and cycling rates in pure stands or in mixtures with other species (Binkley et al., 1992, 1994; Rothe et al., 2002), but these organisms may also affect other soil properties, such as soil P fractions and P cycling (Giardina et al., 1995; Binkley et al., 2000; Rodríguez et al., 2007). As suggested by McKey (1994), leguminous

* Corresponding author. Tel.: +34 954349535; fax: +34 954349391.

E-mail address: xandrouva@gmail.com (A. Rodríguez).

Table 1

Location and soil physical and chemical characteristics of the two studied pine forest stands.

	Location	Altitude (m)	Sand (%)	Silt (%)	Clay (%)	pH soil	Total C (%)	Total N (%)	C:N ratio
Loamy soil	28°47' 5"N 17°55' 52"W	1215	50	30	20	6.7	2.34	0.46	5.12
Sandy soil	28°34' 21"N 17°51' 25"W	1275	95	5	0	6.7	0.86	0.08	10.27

plants require higher concentrations of N than plants in other families, which is not dependent on the N assimilation methods of individual plants. Thus, when atmospheric-N fixation is not possible, such as during P limitation or decreased water availability, leguminous plants must increase N uptake from soil (Sprent and Sprent, 1990; Peoples and Craswell, 1992). Thus, leguminous plants should have an important role in the spatial distribution of soil N fractions, potentially affecting other soil nutrients as well (Prescott, 2002; Koutika et al., 2005).

Texture greatly influences organic matter and water retention capacity (Wardle, 1992; Fisher and Binkley, 2000). The higher diffusion rate of sandy soils results in faster cycling of soil organic matter and water fluxes than in loamy or clay soils. Furthermore, sandy soils are also characterized by a low cation exchange capacity (CEC), which essentially depends on the soil organic matter content. Nevertheless, CEC in loamy and clayed soils is based on both the soil organic matter content and secondary minerals, such as clays (Schlesinger, 1997). Therefore, soil texture may play an important role in regulating the effects of legume individuals on the spatial distributions of N and P in soil, ultimately influencing the spatial structure of plant communities (Kwon et al., 2007). However, no previous study has evaluated the effects of soil texture on the spatial pattern and scale of soil variables.

The primary goal of this study was to investigate the effects of a legume canopy on the spatial pattern and scale of organic-N fractions (microbial biomass-N [MB-N] and dissolved organic-N [DON]) and inorganic-N and -P fractions ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) in two pine forest stands of the Canary Islands (Spain) characterized by contrasting soil textures. These forests are typically N-limited (Tausz et al., 2004; Durán et al., 2008), contributing to the known nutrient composition of the Canary Islands as one of the inhabited areas on Earth with the lowest anthropogenic atmospheric-N depositions (Galloway et al., 2008). Consequently, both inorganic and labile organic-N forms (as DON) may be relevant for plant nutrition because plants and microorganisms may compete for all of these N fractions in N-poor systems (Schimel and Bennett, 2004; Jones and Willett, 2006; Rodríguez et al., 2007). Therefore, we hypothesized that the presence of a legume canopy will more greatly influence the spatial structure of soil N, which is predominantly cycled through biological processes, than P, which is retained by both biological and geochemical mechanisms. Furthermore, the effect of the legume canopy on the spatial structure of the measured N fractions might be modulated by soil texture and the availability of other soil resources. Thus, the local effects of leguminous plants on soil should be more significant for nutrient-poor sandy soils than nutrient-rich loamy soils. Moreover, the spatial pattern should differ during the wet (winter) and the dry seasons (summer) because the microbial processes driving nutrient cycling are sensitive to water availability.

2. Methods

2.1. Area of study

This study was performed on La Palma Island (Canary Islands, Spain, 28° 41' N, 17° 45' W) in two pine forest stands, which are located at

Table 2

Mean and standard error for all soil properties measured in the two different textured soils.

	Loamy soil		Sandy soil	
	Mean (SE)	N	Mean (SE)	N
<i>Winter sampling (wet)</i>				
Soil moisture (%)	20.76 (0.47)	89	1.70 (0.15)	121
MB-N ($\text{mg kg}^{-1}\text{soil}$)	35.19 (2.11)	86	6.61 (0.36)	117
DON ($\text{mg kg}^{-1}\text{soil}$)	3.35 (0.31)	82	1.55 (0.15)	117
$\text{NH}_4\text{-N}$ ($\text{mg kg}^{-1}\text{soil}$)	18.00 (1.24)	88	1.82 (0.29)	121
$\text{NO}_3\text{-N}$ ($\text{mg kg}^{-1}\text{soil}$)	4.64 (0.30)	83	1.92 (0.22)	121
$\text{PO}_4\text{-P}$ ($\text{mg kg}^{-1}\text{soil}$)	6.30 (0.39)	89	5.60 (0.28)	121
<i>Summer sampling (dry)</i>				
Soil moisture (%)	8.27 (0.26)	88	0.28 (0.02)	86
MB-N ($\text{mg kg}^{-1}\text{soil}$)	10.12 (0.90)	78	4.27 (0.41)	82
DON ($\text{mg kg}^{-1}\text{soil}$)	14.31 (0.54)	85	3.43 (0.29)	82
$\text{NH}_4\text{-N}$ ($\text{mg kg}^{-1}\text{soil}$)	4.39 (0.29)	85	2.58 (0.20)	87
$\text{NO}_3\text{-N}$ ($\text{mg kg}^{-1}\text{soil}$)	1.04 (0.07)	83	1.33 (0.18)	85
$\text{PO}_4\text{-P}$ ($\text{mg kg}^{-1}\text{soil}$)	3.02 (0.11)	86	2.87 (0.12)	88

altitudes of 1200–1300 m and are characterized by the same climate and vegetation characteristics, but different soil physical and chemical characteristics (Table 1). High elevations in the Canary Islands are under the influence of a Mediterranean-type climate, characterized by hot, dry summers and cold, wet winters (Font, 2007). Mean annual precipitation and temperature were about 600 mm and 16 °C, respectively (Climent et al., 2004). Pine forest stands with different soil textures are easily located in this plant community due to the different soil ages formed from volcanic eruptions during different times of the island's geologic history. Soils of the two pine forest stands are derived from the weathering of volcanic basaltic rock, but differed in age and therefore in soil texture, as loamy compared to sandy soil. Loamy soil (Leptic Umbrisol) is an old soil characterized by a relative high water-holding capacity, which alleviates water deficiency during the dry season. Sandy soil (Regosol) is a relatively young soil characterized by an incipient A horizon and low water-holding capacity (FAO, 1996). *Pinus canariensis* Chr. Sm. ex DC is an endemic pine of the Canary Islands and is the most abundant forest community on this island, presently covering almost 80% of the soil surface. Under the pine canopy, the understory is sparse and composed of *Adenocarpus viscosus* (Wild.) Webb & Berthel, *Erica arborea* L., and *Cistus symphytifolius* Lam. The leguminous *A. viscosus* is an endemic shrub of the Canary Islands that has the ability to fix

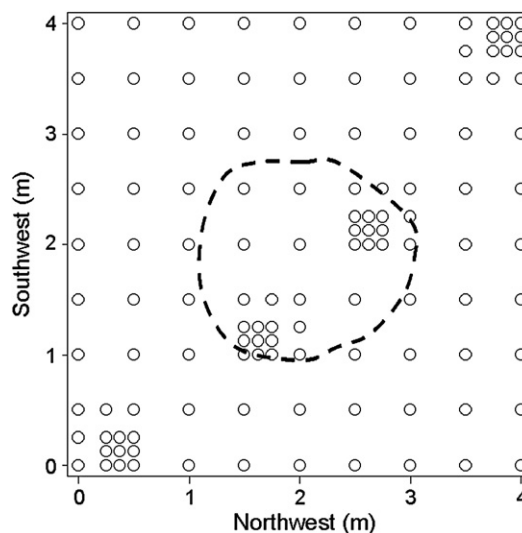


Fig. 1. Winter sampling design for an *A. viscosus* individual in sandy soil. Each circle represents a sampling point, and the dotted line represents the legume canopy projection.

atmospheric-N due to the symbiotic relationship with *Rhizobium* bacteria. Frequently, *A. viscosus* is the only shrub accompanying *P. canariensis* in the pine forest stands of La Palma Island.

2.2. Experimental design

Samplings were performed in late winter (March 2005), characterized by the highest water availability, and in the mid-summer (August 2005), characterized by the lowest water availability (Table 2). For both soil samplings, an isolated mature individual of *A. viscosus* was selected from both the loamy soil and the sandy soil, with a square plot constructed around each individual. In the winter soil sampling, selection of leguminous plants was randomly performed among those individuals with minimal pine influence beneath an open canopy. For the summer soil sampling, different isolated individuals were selected in close proximity to the winter-sampled plants, in order to avoid previously disturbed soils. All selected individuals had similar canopy sizes (1.5–2 m) and heights (ca. 1.5 m),

and the sampled plots were homogeneous in terms of slope (<5%) and soil rock cover. Plot dimensions depended on the size of the individual plant inside the plot and were chosen to maximize the spatial detection of soil properties around individual plants. Thus, the winter-sampled plot in the sandy soil was 4 m × 4 m, while all other plots were 3 m × 3 m. Soil samples were collected from the top 10 cm of the soil profile at 50 cm intervals with a metallic cylinder of 5 cm diameter × 15 cm high. Within each plot, soils were sampled on a smaller scale by randomly selecting four 50 cm × 50 cm squares, collecting samples at 12.5 cm and 25 cm intervals (Fig. 1). The total number of soil samples was 121 from the winter-sampled plot in the sandy soil and 89 from all other plots. Samples were immediately placed in an ice-filled cooler and transported to the lab.

2.3. Laboratory analysis

All soil samples were sieved (<2 mm mesh size) in field-moist conditions, and sub-samples were oven-dried at 80 °C for 48 h to

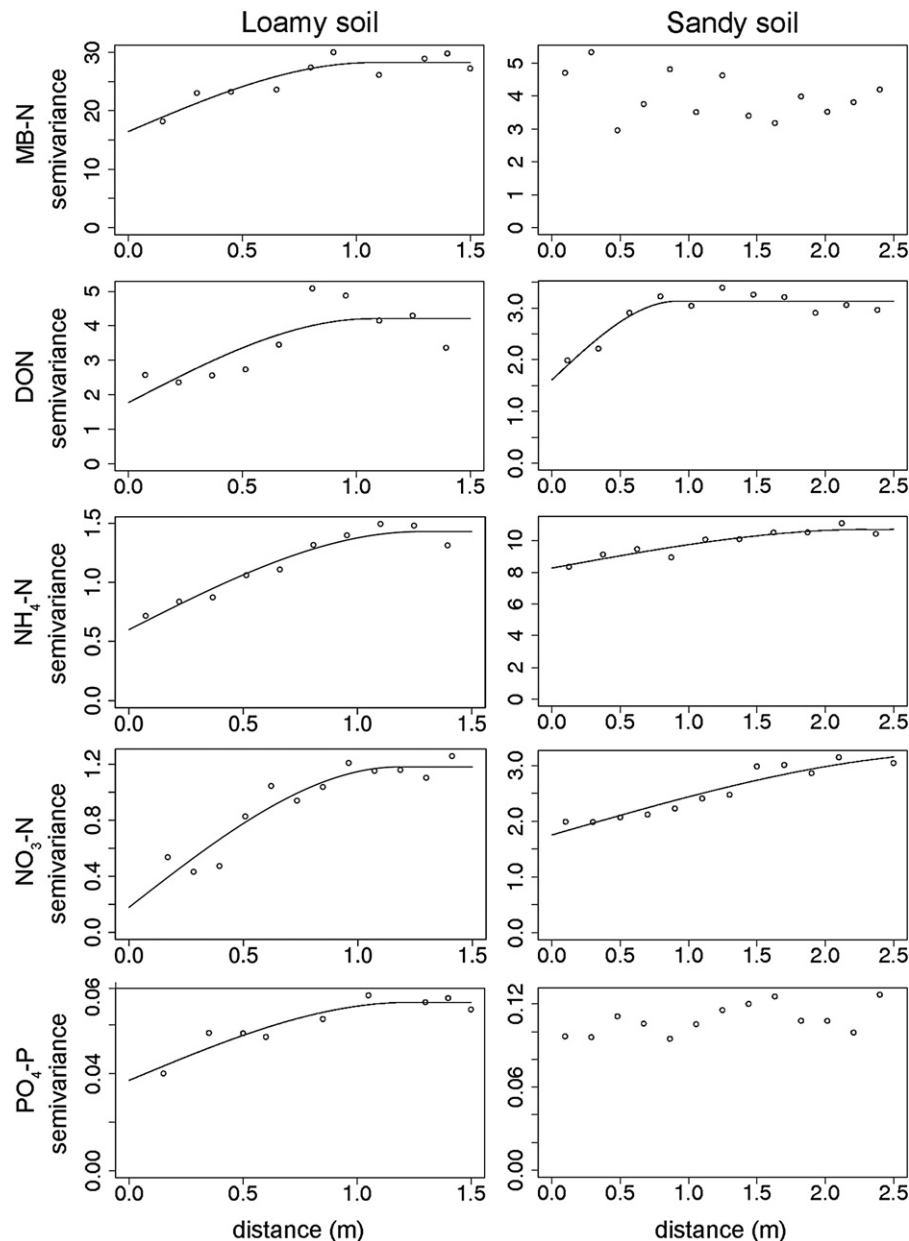


Fig. 2. Semivariograms for all soil properties measured in the loamy and sandy soil of the winter sampling. All variables were expressed as mg kg⁻¹ soil.

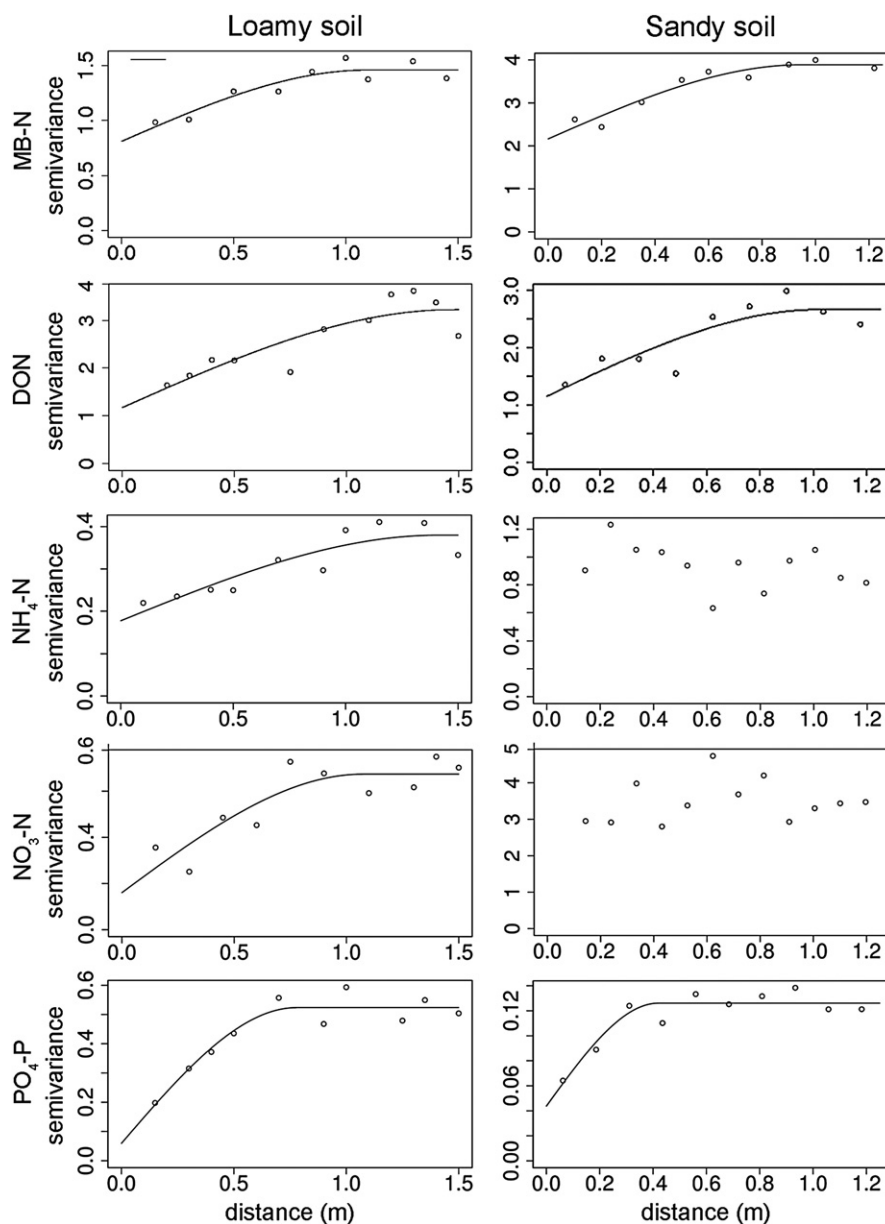


Fig. 3. Semivariograms for all soil properties measured in the loamy and sandy soil of the summer sampling. All variables were expressed as mg kg^{-1} soil.

calculate soil moisture. To analyze MB-N, soil sub-samples (5 g of fresh soil) were fumigated with chloroform for 5 days, and other non-fumigated sub-samples served as the controls. Fumigated and non-fumigated soil sub-samples were extracted with 50 ml of 0.5 M K_2SO_4 (Brookes et al., 1985). Total N in these extracts was estimated via a persulfate oxidation technique, wherein total N was oxidized to $\text{NO}_3\text{-N}$ (D'Elia et al., 1977). The $\text{NO}_3\text{-N}$ concentration in these digests was reduced to ammonium and analyzed by colorimetry (indophenol blue method) in a microplate reader (Sims et al., 1995). Finally, total-N concentration from non-fumigated samples was subtracted from fumigated samples and divided by the fraction of microbial-N extracted after CHCl_3 fumigation ($K_n = 0.54$, Joergensen and Mueller, 1996). Soil DON was analyzed by subtracting mineral-N from total N in the non-fumigated soil sub-sample extracts (Cabrera and Beare, 1993; Doyle et al., 2004). Mineral-N was extracted from 5 g of each fresh soil sub-samples with 50 ml of 2 M KCl by shaking for 1 h at 200 rpm in an orbital shaker, and the suspension was then filtered through a 0.45 μm Millipore filter. As previously described, the amount of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in these extracts was determined by colorimetry. Extractable-P

was estimated following the method described by Nelson and Sommers (1996). Fresh soil sub-samples (2 g) were shaken with 40 ml of 0.5 M NaHCO_3 in an orbital shaker for 1 h at 200 rpm, then filtered through a 0.45 μm Millipore filter and analyzed for $\text{PO}_4\text{-P}$ using a nutrient auto-analyzer (Bran + Luebbe – AA3). Organic and inorganic nutrient pools were expressed in mg kg^{-1} of dry soil.

2.4. Statistical analysis

The spatial pattern and scale of the studied soil N and P fractions were estimated using geostatistical analysis (Robertson, 1987; Rossi et al., 1992; Webster and Oliver, 2001). Prior to geostatistical analysis, all soil properties were transformed to normal distributions according to the formula proposed by Box and Cox (1964):

$$Y' = \log(Y) \text{ if } \lambda = 0,$$

$$Y' = (Y^\lambda - 1) / \lambda \text{ otherwise,}$$

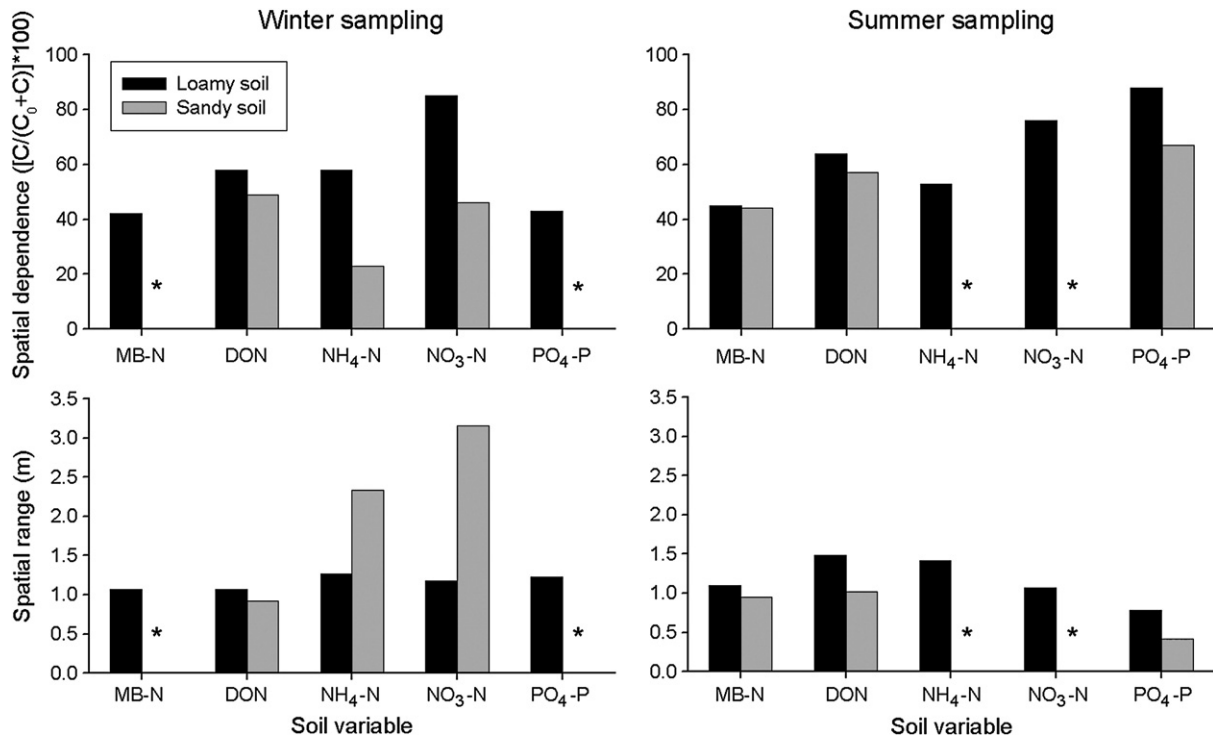


Fig. 4. Spatial dependence and range for all soil properties with spatial structure measured in the two different textured soils for both the winter and summer sampling. (*) = nugget model.

where Y is the variable at the original scale, Y' is the transformed variable, and λ is the transformation parameter.

We used semivariograms to determine the average variance between samples collected at increasing distances from one another (lag interval). To facilitate comparisons, all semivariograms were fitted to a spherical model and the utilization of other models did not significantly improve the fit. To estimate the magnitude of spatial dependence, the percentage of total variance (sill; $C_0 + C$) explained by the structural variance (C , variance explained by spatial autocorrelation) was calculated. Variance occurring on a smaller scale than the field sampling (at 0 lag distance) is known as nugget variance (C_0). A high nugget variance may also indicate sampling or analytical error (Isaaks and Srivastava, 1989). A nugget model would indicate a lack of spatial dependence for the studied scale. The spatial range indicates the geographic scale at which samples show spatial dependence. The model fitted to the semivariogram allows for interpolation ("kriging"), which provides optimal and unbiased estimates of non-sampled points. The interpolation of points using semivariograms (kriging) requires the assumption of stationarity (Webster, 2000; Corstanje et al., 2008), and data were transformed and detrended as necessary (Legendre and Fortin, 1989; Bruckner et al., 1999).

All geostatistical analyses were performed with R 2.7.2 for Linux (R Development Core Team, 2008), using the geoR and gstat modules (Pebesma and Wesseling, 1998; Ribeiro and Diggle, 2001).

3. Results

MB-N, DON, and NH₄-N exhibited higher values in the loamy soil than in the sandy soil (Table 2). NO₃-N only displayed higher values in the loamy soil than in the sandy for the winter sampling, while PO₄-P exhibited similar values between the two soils for both sampling dates.

In the loamy soil, all the empirical semivariograms were successfully fitted to a spherical model, indicating spatial dependence within this soil ($p < 0.05$, Figs. 2 and 3). However, detection of spatial structure failed for two of the five investigated soil variables for both

sampling dates in the sandy soil. Most soil variables with spatial structure demonstrated a greater spatial dependence in the loamy than sandy soil, with the exception of MB-N during summer (Fig. 4). The spatial range varied between 0.78–1.48 m in the loamy plots and 0.56–3.15 m in the sandy plots (Figs. 2 and 3). Except for NH₄-N and NO₃-N in winter plots, the spatial range was also lower in the sandy than the loamy soil (Fig. 4).

Only kriged maps for the sandy soil demonstrated an apparent effect of the legume canopy on the spatial pattern of winter NH₄-N, NO₃-N, and DON, with spatial ranges matching the legume canopy diameter (Fig. 5).

4. Discussion

The legume canopy had a clear effect on the spatial pattern of some measured soil N fractions; however, no dependence was observed for PO₄-P on the legume canopy. The mechanisms retaining these two nutrients in soils may explain the difference in the spatial dependence from the plant canopy. N is predominantly cycled through organic matter, and thus, the spatial pattern should be affected by biological processes that are driven by plants (Schlesinger et al., 1996; Hirobe et al., 2003; Gallardo and Paramá, 2007). In contrast, P is cycled through both biological and geochemical mechanisms, due to strong interactions with soil minerals, which may lead to complex spatial patterns (Gallardo, 2003b; Grunwald et al., 2004, 2006). The presence of an isolated individual should more intensively modify the biological than the geochemical retention mechanisms, resulting in different spatial properties of soil N and P (Gallardo, 2003a).

As expected, the effect of the legume canopy was more significant in the sandy nutrient-poor soil, which had a total-N content almost six times lower than the loamy soil. Winter DON, NH₄-N, and NO₃-N demonstrated the highest spatial dependence on the plant canopy. A higher concentration of N-rich organic matter from litterfall beneath the legume canopy may explain the higher DON content (Koutika et al., 2005), and the higher NH₄-N and NO₃-N concentrations to some extent because DON represents the substrate that ultimately

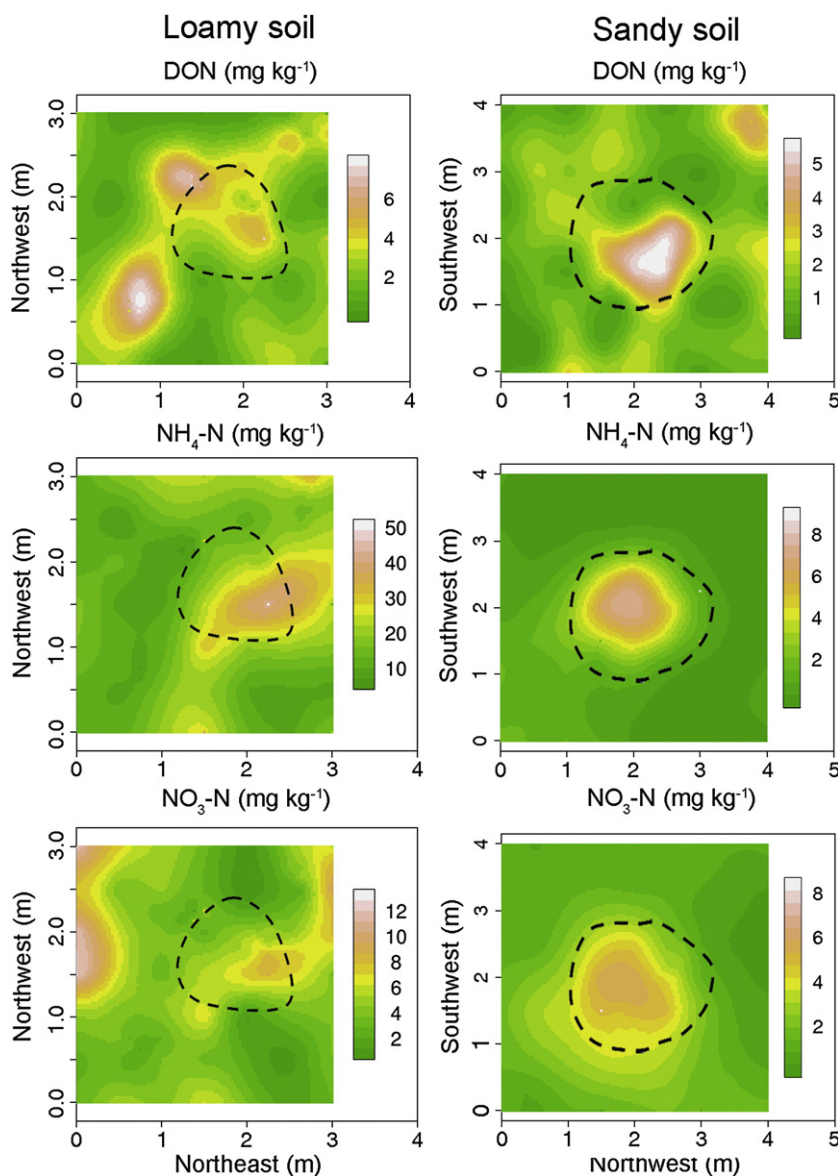


Fig. 5. Interpolation maps (kriging) for DON, $\text{NH}_4\text{-N}$, and $\text{NO}_3\text{-N}$ in the loamy and sandy soil of the winter sampling. The dotted line represents the legume canopy projection.

results in $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in soil (Jones et al., 2005; Christou et al., 2006). In the loamy soil, the lack of spatial correlation between the legume canopy projection and DON, $\text{NH}_4\text{-N}$, and $\text{NO}_3\text{-N}$ may be related to the stabilization of soil organic matter by clays through both mineral–organic matter binding and the physical protection provided by the micropores in clay aggregates (Wattel-Koekkoek et al., 2001). Thus, the spatial distribution of this organic matter may be more related to previous vegetation or other historic processes and less dependent on recently added organic matter (Yankelevich et al., 2006).

The soil MB-N did not show any spatial relationship with the legume canopies, suggesting that this microbial biomass is not a nitrogen sink under the plant canopy and may instead be a source for DON and inorganic-N under leguminous plants (Jones et al., 2005). Interestingly, MB-N exhibited very similar spatial structure in both soils, suggesting that the same process may determine the spatial pattern of this soil variable.

As indicated in previous studies, the spatial pattern changed rapidly over time and differentially for each soil variable (Gross et al., 1995; Ryl et al., 1996; Cain et al., 1999; Guo et al., 2002). For example, the spatial

dependence of DON increased from winter to summer for both soil types. These increases were coincident with a higher soil DON concentration during summer, which was likely a result of the soil microbial biomass decomposition. Thus, the new DON spatial structure would be related to the former MB-N spatial structure. Inversely, both the higher N mineralization and DON uptake rates by soil microorganisms during the wet season would mainly decrease the soil DON concentration at sites with the highest DON content, and thus, decrease the spatial pattern intensity (Rodríguez et al., 2009).

Drastic changes were observed for soil $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in the sandy soil, disappearing in summer the spatial dependence from the legume canopy previously observed in winter. The loss of spatial structure supported our hypothesis regarding differences in the spatial pattern under high and low water availability. Environmental stress, such as limited water availability, affects the N-fixing process more significantly than N assimilation and uptake (Streeter, 1994). Consequently, the lower water content in the sandy soil may have diminished soil mineralization and N-fixation rates more than N uptake, resulting in a decrease of soil N from the highest concentration patches (Wang et al., 2007). For instance, Wienhold and Klemmedson

(1991) observed a higher dependency of soil N by nodulated plants under water stress. The fade of the winter spatial structure in summer was not detected in the loamy soil, perhaps due to the protective effect of clays against nodule desiccation (Marshall, 1964).

Although these mechanisms may explain the noted differences in spatial patterns between summer and winter soil samples, this conclusion is not definitive since the temporal replicates of this study were performed on different legume individuals, albeit in close proximity. In addition, the high number of soil samples needed to characterize the spatial properties in one soil plot restricted the collection of samples from more than one individual per soil type. Inconsistencies between replicate plots and temporal changes observed in spatial patterns during other investigations suggest that conclusions based on data from a single plot or a single sampling date should be interpreted with caution (Robertson et al., 1997; Guo et al., 2002).

The spatial dependence found in this study was highly variable across soil variables, soil texture, and sampling dates, but with values ranging similarly to other studies. For example, Jackson and Caldwell (1993b) found spatial dependence between 34% and 93% for different soil variables using a similar sampling design. The spatial ranges found in our study were also similar to ranges indicated in previous studies that were performed on the same spatial scale (Palmer, 1990; Lechowicz and Bell, 1991; Gross et al., 1995; Gallardo and Paramá, 2007). Fine-scale heterogeneity in these previous studies was suggested to be derived from the effects of individual plants on nutrient availability through differences in stemflow, throughflow, litterfall, or litter decomposition. However, our results only detected an apparent individual effect of the legume canopy for one of the temporal replicates as well as for the sandy soil, indicating that other factors (such as soil texture and soil moisture) may be responsible for this fine-scale heterogeneity. As a general pattern, the spatial dependence was lower in the sandy soil than in the loamy soil, even for those variables clearly influenced by the plant canopy location. Except for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in winter plots, the spatial range was also lower in the sandy than the loamy soil, indicating the importance of the recently added legume litter as a source of soil organic matter in the sandy soil compared to the older organic matter accumulations in the loamy soil.

Our results suggest that the presence of *A. viscosus* individuals may be an important source of spatial heterogeneity for the soil N of these forests. However, soil texture and water content would modulate the magnitude of the legume canopy effect on the spatial distribution of these N forms beneath the canopies. Soil texture is more stable than other biotic and abiotic soil factors, and thus, this parameter may be a potentially useful metric for predicting soil N spatial heterogeneity in these forests (Dupuis and Whalen, 2007). Investigations of the effect of leguminous plants on the nutrient spatial heterogeneity of soil will provide a greater understanding of ecosystem functioning, particularly when the global N cycle has been deeply altered by human influences (Galloway et al., 2008). Therefore, further studies are needed in different ecosystems to understand the effects of legume individuals on the spatial pattern and scale of N resources for plants.

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