

Spatial pattern and variability in soil N and P availability under the influence of two dominant species in a pine forest

Alexandra Rodríguez · Jorge Durán ·
Felisa Coveló · José María Fernández-Palacios ·
Antonio Gallardo

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Abstract The presence of a legume in a nitrogen (N)-limited forest ecosystem may not only create “islands of N fertility” but also affect the phosphorus (P) availability. The main objective of this study was to compare the effect of a pine (*Pinus canariensis*) and a leguminous (*Adenocarpus viscosus*) species on the spatial pattern and variability of different labile organic-N (microbial biomass-N [MB-N] and dissolved organic-N [DON]), as well as inorganic-N (IN) and -P fractions (NH₄-N, NO₃-N, and PO₄-P), in a forest soil of the Canary Islands (Spain). Assuming some litter quantity and quality differences between these two species, we expected to find higher soil labile organic-N concentrations under isolated individuals of *P. canariensis* than under isolated individuals of *A. viscosus*. We also expected to find higher concentrations and spatial dependence (percentage of total variance explained by spatial autocorrelation) of NO₃-N beneath *A. viscosus* than beneath *P. canariensis* canopies, and higher spatial scaling of soil

variables under the influence of *P. canariensis* canopies than under the presence of *A. viscosus* individuals. Moreover, we tested whether the soil variables measured under isolated individuals of both species showed a different spatial variability than the same soil variables measured under overlapping pine canopies inside a pine forest. To test these hypotheses, soil samples under isolated mature individuals of each species were collected in the winter and summer, whereas under a pine forest canopy, the sampling was performed only in the winter. The winter MB-N and DON concentrations were significantly higher beneath the pine individual, whereas the winter NO₃-N, NO₃-N-to-IN ratio, and PO₄-P were significantly higher under the leguminous individual; these differences were not observed in the summer samples. We found higher spatial ranges under the pine than under the legume canopy in the winter sampling, and the spatial dependence of NO₃-N was twice as high beneath the legumes as under the pines at both sampling dates. The soil spatial variability was higher (up to 17 times higher) under isolated individuals than inside the pine forest. The results of this study suggest that both the morphological and physiological characteristics of *P. canariensis* and *A. viscosus*, as well as the spatial pattern of *P. canariensis*, may influence the spatial pattern and variability of soil resources.

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A. Rodríguez (✉) · J. Durán · F. Coveló · A. Gallardo
Department of Physics, Chemical and Natural Systems,
Pablo de Olavide University,
Seville 41013, Spain
e-mail: xandrouva@gmail.com

J. M. Fernández-Palacios
Department of Parasitology, Ecology and Genetics,
La Laguna University,
La Laguna 38207, Spain

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Introduction

The availability of nitrogen (N) in the soil directly influences a wide range of ecological processes, both above and below ground, at the physiological, community, ecosystem, and global levels (Frank and Groffman 2009). Several factors tend to reduce the biological availability of N in temperate ecosystems, such as the strong link between organic-N and recalcitrant carbon compounds in soils, as well as the mobility of N out of the ecosystems by hydrological and atmospheric pathways (Vitousek et al. 2002). Thus, this essential nutrient for plants frequently constrains net primary production in most temperate terrestrial ecosystems (Vitousek and Howarth 1991; Elser et al. 2007), and both N input and availability become key factors regulating the organization and metabolism of many of these ecosystems (Boring et al. 1988).

Pines in the *Pinus canariensis* forests are frequently accompanied by the presence of the legume *Adenocarpus viscosus*, but the role of this latter species in this ecosystem is not well understood (Rodríguez et al. 2009b). Leguminous plants require higher concentrations of N than plants from many other families (McKey 1994), and they increase N uptake from the soil when the atmospheric-N fixation is not sufficient (Sprent and Sprent 1990; Peoples and Craswell 1992). Thus, the presence of a legume may create “islands of N fertility” in these ecosystems through the return of N to the soil with the leaf litter fall, root turnover and root exudation (Binkley et al. 1992; Prescott 2002). Moreover, most N-fixing plants also have high requirements for P (Sprent 1988). Therefore, legumes play an important role in the spatial pattern and variability of N and P fractions in soil (Prescott 2002; Koutika et al. 2005).

The spatial and temporal heterogeneity is a dominant and conspicuous feature of terrestrial ecosystems (Bruckner et al. 1999; Quilchano et al. 2008). Identifying the spatial pattern and scale of soil resources is important to avoid the negative influence of the spatial autocorrelation when designing field experiments. Similarly, understanding the causes of such spatial patterns and their consequences on the process at the individual, population, and community levels is crucial to sustainably manage and preserve soil resources (Hutchings et al. 2003; Legendre et al. 2004). In forest ecosystems, we may find spatial

patterns of soil properties related to the location of trees (Zinke 1962). In turn, these patches are important local nutritional reserves that not only influence the morphological and physiological properties of plants (Antonovics et al. 1987) but also the coexistence and diversity of species (Schlesinger et al. 1990), and the competitive interactions between individuals (Anderson et al. 2004). Furthermore, the spatial pattern and the scale of soil resources vary temporally, even within a single growing season, and each soil resource can vary in different ways (Guo et al. 2002; Gallardo and Covelos 2005). Thus, plants must acquire soil resources that change in time and space, but also nutrients that exhibit temporal changes in their spatial pattern and scale (Ryel et al. 1996). By considering this variability, we gain another fundamental dimension of the system behavior and an improved ability to identify the organizing features of ecosystems (Fraterrigo and Rusak 2008). In earlier studies, variability was considered an important attribute of ecosystems only in association with the mean. However, recent studies recognize that investigating this variability can capture effects not detected or obscured by averaging (Fraterrigo and Rusak 2008).

In this study, we aim to compare the effect of a pine (*Pinus canariensis*) and a leguminous (*Adenocarpus viscosus*) species on the spatial pattern and variability of labile organic-N fractions, as well as inorganic-N and -P fractions, in a forest soil at La Palma (Canary Islands, Spain). These forests are one of the inhabited areas on Earth with the lowest anthropogenic atmospheric-N depositions (Galloway et al. 2008) and are typically N-limited (Tausz et al. 2004; Durán et al. 2008). In these N-poor systems, both inorganic- and labile organic-N forms may be relevant for plant nutrition because plants and microorganisms may compete for both of these N fractions (Schimel and Bennett 2004). We assumed a higher litter input to the soil from the pine (an evergreen species that can reach more than 60 m height) than from the legume (a semi-deciduous shrub that rarely exceeds one meter height). We also assumed a higher litter quality from the legume than from the pine, whose needles typically show low N concentrations (Tausz et al. 2004, Durán et al. 2009, Morales 2010). Accordingly, we expected to find: 1) higher soil labile organic-N concentrations beneath isolated individuals of *P. canariensis* than under isolated individuals of *A. viscosus*; 2) higher

concentrations and spatial dependence (percentage of total variance explained by spatial autocorrelation) of $\text{NO}_3\text{-N}$ beneath *A. viscosus* than beneath *P. canariensis* canopies; and 3) a higher spatial scaling of soil variables under the influence of *P. canariensis* individuals than under the presence of the small *A. viscosus* individuals. Furthermore, we also tested whether the soil variables measured under isolated individuals of both species showed a different spatial variability than the same soil variables measured under overlapping pine canopies inside a pine forest.

Methods

Area of study

The present study was carried out in one of the two *Pinus canariensis* forest stands used in two previous studies (Rodríguez et al. 2009a, b). This stand is located on the northwest face of La Palma Island, at an altitude of 1,200–1,300 m (Canary Islands, Spain, 28° 41' N, 17° 45' W), under the influence of a Mediterranean-type of climate, which is characterized by hot-dry summers and cold-wet winters (Font 2007). The mean annual precipitation and temperature are about 600 mm and 16°C, respectively (Climent et al. 2004). The soil (Leptic Umbrisol) is derived from the weathering of volcanic basaltic rock and is characterized by a relative high water-holding capacity, which alleviates the water deficiency during the dry season (FAO 1996). The soil organic matter content is about 4%, and the soil pH oscillates between 6.5 and 7. The species *Pinus canariensis* Chr. Sm. ex DC is an endemic pine of the Canary Islands, and forests mainly composed of this species (60-to-70% of pine canopy cover, Méndez 2010) are the most abundant communities on La Palma, presently covering almost 80% of the area. Under the pine forest canopy, the understory is sparse and composed of *Adenocarpus viscosus* (Wild.) Webb & Berthel, *Erica arborea* L. and *Cistus symphytifolius* Lam. The species *A. viscosus* is an endemic leguminous semi-deciduous shrub of the Canary Islands with the ability to fix atmospheric-N due to its symbiotic relationships with *Rhizobium* bacteria; frequently, it is the only shrub accompanying *P. canariensis* in the pine forest stands of La Palma Island.

Experimental design

We re-analyzed some of the samples collected by Rodríguez et al. (2009a, b) to compare the effect of isolated mature individuals of *Pinus canariensis* and *Adenocarpus viscosus* on the spatial pattern and variability of labile N and P fractions. We considered as isolated individuals those that were at a distance of at least five times their canopy radius from other individual plants. We selected isolated individuals as close as possible to the pine forest in order to ensure similar soil and microclimate conditions. However, our study design does not allow us to rule out the possibility of pre-existing factors determining the distribution of our isolated pine and legumes. In these two studies, a total of eight square plots were established around individuals of *P. canariensis* (pine plots, Rodríguez et al. 2009a) and *A. viscosus* (legume plots, Rodríguez et al. 2009b) in two contrasting soil textures. For testing our new hypotheses, we chose the two pine and the two legume plots established in the loamy soil as all of them were in the same stand. We also used new data from pine forest plots to test whether the soil variables measured under isolated individuals of both species showed a different spatial variability than inside the pine forest.

For the isolated individuals, soil sampling was carried out in late winter (March 2005) and in mid-summer (August 2005) with the highest and lowest water availability, respectively (Tables 1 and 2). The sampled individuals of each species had similar canopy size and height. In general, *P. canariensis* individuals had 3–4 m of canopy diameter and ca. 20 m of height, and *A. viscosus* individuals had a canopy diameter and a height of approximately 1.5 m (Fig. 1). All the sampled plots were homogeneous in terms of slope (< 5%) and soil rock cover, and there were no herbaceous cover or other plants in any of them besides the target individual. The plot dimensions depended on the size of the individual plant inside each plot and were chosen to maximize the spatial detection of the soil properties around each individual plant (Fig. 1). After removing the leaf litter layer, we collected soil samples from the top 10 cm of the soil profile at regular intervals using a 15×5 cm metallic corer. In addition, within each plot, the soil was sampled at a smaller scale by randomly selecting four squares (Fig. 1). A total of 121 soil samples were collected from the summer pine plot, and 89 from each of the other plots.

Table 1 Mean (standard error) of all the soil variables measured in the winter samples. Results of the comparison between the samples collected under the isolated pine (P) and legume (L), and under the isolated individuals and the pine forest canopy (PF)

				One-way ANOVA		Permutation Test	
	P N=89	L N=89	PF N=4	P vs. L F	p	P vs. PF p	L vs. PF p
Moisture (%)	24.41 (0.42)	20.76 (0.47)	27.34 (1.72)	1.24	0.267	0.089	< 0.050
MB-N (mg kg ⁻¹)	56.91 (2.51)	35.19 (2.11)	63.58 (7.03)	43.69	0.000	0.337	< 0.010
DON (mg kg ⁻¹)	5.34 (0.30)	3.35 (0.31)	34.38 (1.09)	30.09	0.000	< 0.001	< 0.001
NH ₄ -N (mg kg ⁻¹)	18.88 (1.64)	18.00 (1.24)	29.46 (4.89)	24.23	0.000	0.111	0.295
NO ₃ -N (mg kg ⁻¹)	4.21 (0.34)	4.64 (0.30)	10.19 (1.06)	14.84	0.000	< 0.001	< 0.001
PO ₄ -P (mg kg ⁻¹)	4.79 (0.20)	6.30 (0.39)	6.56 (1.21)	22.43	0.000	< 0.050	0.566
IN-to-PO ₄	4.23 (0.30)	4.16 (0.27)	6.73 (1.33)	3.09	0.081	< 0.050	< 0.050
NO ₃ -to-IN (%)	22.80 (1.92)	23.85 (1.41)	27.06 (5.12)	5.35	0.022	0.291	< 0.050

p values below 0.05 are in bold

For the pine forest stands, we selected four 25 × 25 m plots in the winter sampling, and we collected 15 random samples from the top 10 cm of the soil profile in each plot. To account for as much geographical, geological, and biological variability as possible, the criterion for the selection of these plots was that two of the replicate plots had a gentle slope (4–8%), whereas the other two had a steep slope (20–25%). Similarly, North and South plots were selected. All the soil samples were placed separately inside polyethylene bags and immediately transported inside coolers to the laboratory.

Laboratory analysis

All the soil samples were sieved (< 2-mm mesh size) in field-moist conditions to remove stones, large roots and non-decomposed leaf litter. Then, we measured

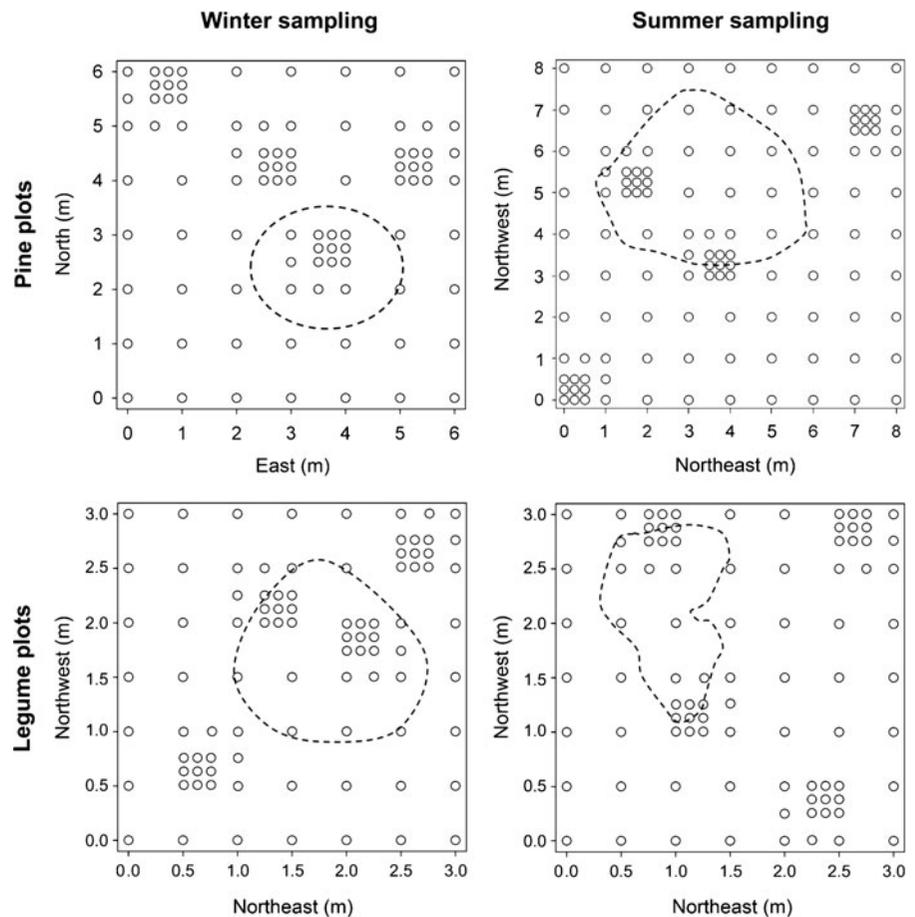
the gravimetric water content by oven-drying subsamples at 80°C for 48 h to calculate the soil variables on a dry weight basis. We estimated the concentration of labile organic-N fractions, such as the microbial biomass-N (MB-N) and the dissolved organic-N (DON), as well as the inorganic-N and -P fractions (NH₄-N, NO₃-N, and PO₄-P; Rodríguez et al. 2009a, b). The concentration of MB-N was determined by the fumigation-extraction method (Brookes et al. 1985), followed by the persulphate oxidation technique (D’Elia et al. 1977) and a colorimetric analysis (indophenol blue method) in a microplate reader (Sims et al. 1995). Soil DON was estimated by subtracting inorganic-N (see below) from total-N in the non-fumigated soil subsample extracts (Cabrera and Beare 1993; Doyle et al. 2004). The inorganic-N (IN) was obtained by extracting the NH₄-N and the NO₃-N from soils with 2 M KCL and colorimetrically

Table 2 Mean (standard error) of all the soil variables measured in the summer samples. Results of the comparison between the samples collected under the isolated pine (P) and the isolated legume (L)

p values below 0.05 are in bold

	One-way ANOVA			
	P N=121	L N=89	P vs. L F	p
Moisture (%)	6.24 (0.13)	8.27 (0.26)	0.10	0.747
MB-N (mg kg ⁻¹)	12.08 (0.69)	10.12 (0.90)	3.63	0.058
DON (mg kg ⁻¹)	12.12 (0.29)	14.31 (0.54)	47.40	0.000
NH ₄ -N (mg kg ⁻¹)	3.60 (0.19)	4.39 (0.29)	37.05	0.000
NO ₃ -N (mg kg ⁻¹)	1.33 (0.05)	1.04 (0.07)	3.80	0.053
PO ₄ -P (mg kg ⁻¹)	3.39 (0.16)	3.02 (0.11)	0.03	0.857
IN-to-PO ₄	1.71 (0.10)	1.90 (0.12)	3.07	0.081
NO ₃ -to-IN (%)	30.93 (1.29)	22.15 (1.80)	11.99	0.001

Fig. 1 Sampling design beneath the isolated individuals of *P. canariensis* (pine plots) and *A. viscosus* (legume plots) in the winter and summer sampling dates. Each circle shows a sampling point, and the dotted line represents the tree or shrub canopy projection



determined (indophenol blue method) in a microplate reader (Sims et al. 1995). Finally, we estimated the extractable-P ($\text{PO}_4\text{-P}$) by following the method described by Nelson and Sommers (1996) and using a Bran + Luebbe-AA3 auto-analyzer. All of the organic and inorganic nutrient fractions were expressed in mg kg^{-1} of dry soil.

Statistical analysis

We used a one-way ANOVA with species as a fixed factor to evaluate the differences between the plots of the two species considering all the soil variables and a permutation test to compare the winter samples collected under the pine forest canopy and under isolated individuals (Röhmel 1996). Before performing the ANOVA and the permutation test, we removed the spatial autocorrelation effect between samples in all the analyzed soil variables in plots of isolated individuals using a conditional autoregressive model (CAR, Cressie

1993). This model assumes that the dependent variable value in each location is a function of both the explanatory variable and the values of the dependent variable at neighboring locations (Lichstein et al. 2002). Prior to the ANOVA, we also tested whether either untransformed or log-transformed variables satisfied the normality and variance homogeneity assumptions (Shapiro-Wilk and Levene tests, respectively).

The estimate of the spatial pattern and scaling of the studied soil N and P fractions was performed by geostatistical analyses using semivariograms to determine the average variance between samples collected (semivariance) at increasing distances from one another (Robertson 1987; Rossi et al. 1992; Webster and Oliver 2001). Because the utilization of other models did not significantly improve the fit (measured through the coefficient of determination [R^2]), all semivariograms were fitted to a spherical model to facilitate the comparisons. We estimated the magnitude of spatial dependence by calculating the percentage of total

variance (structural variance + nugget variance) explained by the structural variance (variance explained by spatial autocorrelation). Nugget variance is known as the variance that occurs on a smaller scale than the field sampling. When the value of spatial dependence approaches 100%, the semivariogram first rises for comparisons of neighboring samples that are similar and autocorrelated, and then levels off indicating the distance (spatial range) beyond which samples are independent. When the spatial dependence is close to 0% (randomly distributed data), there is a little change in the semivariance with increasing distance, and the variogram remains flat. We designated as “nugget model” this model where no spatial dependence between samples was detected for the considered scale. The spatial range indicated the geographic scale at which the samples showed a spatial dependence.

We estimated the spatial variability of all the soil variables by calculating both the standard deviation of the log-transformed variables (SDL) and the coefficient of variation (CV, Fraterriigo and Rusak 2008). All statistical analyses were performed with R 2.7.2 for Linux (R Development Core Team 2008), using the “exactRankTests” package for the permutation test (Hothorn and Hornik 2006), the “spdep” package for the conditional autoregressive model (Bivand et al. 2005), and the “geoR” and “gstat” packages for the geostatistical analyses (Pebesma and Wesseling 1998, Ribeiro and Diggle 2001).

Results

In the winter samples, the soil MB-N, DON, and $\text{NH}_4\text{-N}$ were significantly higher in the pine than in the legume plot, while the $\text{NO}_3\text{-N}$, the $\text{NO}_3\text{-to-IN}$ ratio, and the $\text{PO}_4\text{-P}$ were significantly higher in the legume plot (Table 1). However, these differences between the pine and the legume plot disappeared or the results were the opposite in the summer samples (Table 2). The pine forest showed the highest values of soil DON, $\text{NO}_3\text{-N}$, and IN-to-PO_4 ratio, and higher values for $\text{PO}_4\text{-P}$ and for MB-N and the $\text{NO}_3\text{-to-IN}$ ratio than the pine and legume plots, respectively (Table 1).

In the legume plots of both winter and summer sampling, all of the empirical semivariograms were predicted by a spherical model ($P < 0.05$), indicating a spatial dependence of the soil variables at the plot spatial scale (Fig. 2). However, in the pine plots, the

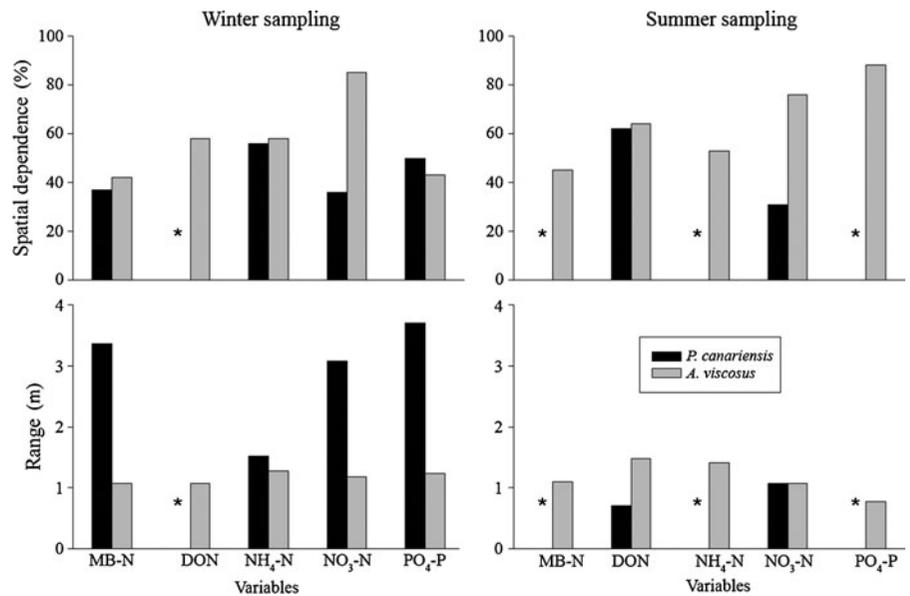
detection of a spatial structure failed for the winter DON and for the summer MB-N, $\text{NH}_4\text{-N}$, and $\text{PO}_4\text{-P}$ (Fig. 2). In the winter sampling, all the variables with spatial structure had a higher spatial range in the pine than in the legume plot, but this result was not observed in the summer sampling (Fig. 2). The spatial dependence values were similar between both species in both sampling dates with the exception of $\text{NO}_3\text{-N}$, whose spatial dependence was twice as high in the legume as in the pine plots (Fig. 2).

The pine forest soil showed the lowest SDL and CV values for most of the variables measured in the winter samples (Table 3). In both the winter and the summer sampling, the SDL and CV of the IN-to-PO_4 ratio were similar in the isolated individual plots of both species, whereas those statistics of the MB-N and DON were lower in the pine than in the legume plots. No clear trend was observed in the other soil variables (Table 3).

Discussion

The winter sampling results supported our hypotheses about the effect of the two species on the soil nutrient conditions, because there were higher labile organic-N (MB-N and DON) and $\text{NH}_4\text{-N}$ concentrations in the pine than in the legume plot, whereas the legume plot showed the highest values in the variables directly related to the nitrification ($\text{NO}_3\text{-N}$ and $\text{NO}_3\text{-to-IN}$) and in $\text{PO}_4\text{-P}$ (Table 1). The quantity and quality of the leaf litter and organic matter associated with each species could explain some of these differences. The increased productivity of the pines compared to the legumes may result in a higher accumulation of organic matter and soil microbial biomass, leading to both greater decomposition and depolymerization rates, which increases the dissolved organic-N concentrations (Jones et al. 2005). On the other hand, a high C-to-N ratio in the leaf litter (typical of pine litter) could provide enough C to favor heterotrophic microbial growth ($\text{NH}_4\text{-N}$ immobilization) instead of an autotrophic metabolism ($\text{NH}_4\text{-N}$ oxidation; Robertson 1982). However, with a relatively low C-to-N ratio (expected in legume litter), the heterotrophic microbes may be temporarily C-limited during the late decomposition stage, allowing the autotrophic nitrifiers to have an advantage by converting $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$. These differences in the soil microbial community may also explain the higher

Fig. 2 Spatial dependence and range of all the soil variables with the spatial structure found in the pine and the legume plots for winter and summer sampling dates. (*) = nugget model



MB-N concentration found under the pine than under the legume individuals (Rodríguez et al. 2007).

Symbiotic N-fixers require more P than non-fixers; legumes can meet this larger requirement by releasing P from unavailable sources in a variety of ways, such as increasing phosphatase activity (Sprent 1988). Thus, higher soil P availability and a greater return of P to the soil through leaf litter associated with the presence of *A. viscosus*, rather than with the presence

of *P. canariensis*, could explain the highest soil PO₄-P concentration found in our legume plot (Table 1). The presence of N₂-fixing species appeared to increase the soil available-P in other studies (Giardina et al. 1995; Zou et al. 1995). However, no consistent pattern appears to exist, because some studies have reported that the presence of N₂-fixing species did not appear to increase the soil P availability (Cole et al. 1991, Rodríguez et al. 2007).

Table 3 Standard deviation of the log-transformed variables (SDL) and coefficient of variation (CV) of all the soil variables measured in both sampling dates under the isolated individuals (P = pine, L = legume) and the pine forest canopy (PF)

Variable	Sampling date	P		L		PF	
		SDL	CV	SDL	CV	SDL	CV
Moisture (%)	Winter	0.07	16	0.10	21	0.05	13
	Summer	0.09	23	0.11	30	n.d.	n.d.
MB-N (mg kg ⁻¹)	Winter	0.22	41	0.32	56	0.08	19
	Summer	0.29	60	0.32	78	n.d.	n.d.
DON (mg kg ⁻¹)	Winter	0.21	51	0.34	84	0.02	5
	Summer	0.10	25	0.15	35	n.d.	n.d.
NH ₄ -N (mg kg ⁻¹)	Winter	0.38	81	0.27	65	0.14	33
	Summer	0.18	56	0.20	62	n.d.	n.d.
NO ₃ -N (mg kg ⁻¹)	Winter	0.27	73	0.21	58	0.08	21
	Summer	0.10	40	0.15	63	n.d.	n.d.
PO ₄ -P (mg kg ⁻¹)	Winter	0.13	38	0.19	59	0.14	37
	Summer	0.18	51	0.11	33	n.d.	n.d.
IN-to-PO ₄	Winter	0.23	62	0.19	60	0.17	39
	Summer	0.14	62	0.14	58	n.d.	n.d.
NO ₃ -to-IN (%)	Winter	0.30	73	0.22	54	0.15	38
	Summer	0.20	43	0.56	73	n.d.	n.d.

n.d. = no data

None of the differences in the soil variables measured beneath both species in the winter remained in the summer sampling, when a DON increase and a general decrease of the other variables were observed (Tables 1 and 2). During the summer, the low soil moisture may have caused the decline of the microbial biomass involved in organic matter mineralization (Wardle 1992). Both the lower organic matter mineralization, and a lower DON uptake by soil microorganisms (induced by the soil drying), together with the active nutrient uptake by plants during the growing season, would explain the increase in the DON concentration and the decline in the inorganic-N and -P pools in the summer (Vitousek and Matson 1985; Campo et al. 1998). However, the highest DON and $\text{NH}_4\text{-N}$ concentrations found in the legume plot in the summer sampling, as well as the lowest DON and $\text{NH}_4\text{-N}$ but highest $\text{NO}_3\text{-N}$ concentrations found in the winter sampling, suggest greater N input to the soil from the *A. viscosus* leaf litter and faster soil N cycling with appropriate temperature and humidity conditions.

The pine forest had between 7- and 10-times higher DON concentrations as compared with the isolated individuals (Table 1). These differences might be a simple effect of the variation in plant cover between plots, but these differences were much greater than those obtained with other soil variables, such as microbial biomass-N, inorganic-N or $\text{PO}_4\text{-P}$. These results suggest that the overlapping pine canopies in our pine forest plots may have an interactive effect, favoring the accumulation of DON rather than the process leading to the accumulation of mineral forms.

The geostatistical analysis of the winter samples corroborated the hypothesis of a higher spatial scale (range) in the presence of isolated individuals of *P. canariensis* than in the presence of small isolated individuals of *A. viscosus*, decreasing the spatial range proportionally to the plant size (Ettema and Wardle 2002). However, this hypothesis was not supported by the geostatistical analysis of the summer samples, where we observed the loss of the winter spatial structure for most variables in the pine plot but not in the legume plot (Fig. 2). Several studies found deeper temporal differences between semivariograms of variables limiting plant growth than of non-limiting variables (Ryel et al. 1996). Accordingly, the observed reduction of spatial structure in the N fractions

of the pine plots may be explained by the highest N-limitation expected for the pines (non-fixer species). A higher N-uptake rate of the pines compared with that of the legumes in areas with higher concentration may have increased the temporal variability in the spatial pattern of these N fractions (James et al. 2003). Alternatively, the differences in the soil spatial structure stability beneath the canopies of these two species could also be due to inherent characteristics of the leaf litter accumulated under each individual (Quilchano et al. 2008). Finally, these temporal differences also corroborate that, with the exception of the stable pattern found in arid and semi-arid ecosystems (Schlesinger et al. 1996), the range provided by semivariograms could be a weak and very ephemeral indicator of the spatial distribution of available nutrients in many ecosystems.

The highest $\text{NO}_3\text{-N}$ spatial dependence observed in the legume plots of both sampling dates confirmed another of our hypotheses. As explained above, the higher concentration of N-rich organic matter in the presence of *A. viscosus* might favor the nitrification process, increasing both the availability and the spatial structure of $\text{NO}_3\text{-N}$ under this legume.

Similarly, the soil variability estimated through the SDL and CV was higher under the isolated individuals of both species (up to 17 times higher for some variables) than inside the pine forest (Table 3). These differences could be due to the more homogeneous and continuous leaf litter layer and rooting systems beneath the pine forest canopy than beneath isolated individuals. Inside the pine forest, the leaf litter from different individuals could overlap, whereas under the isolated individuals the litter accumulation is determined by the canopy size and shape along with the prevailing wind direction and microtopographic heterogeneity (Robertson et al. 1997). A more heterogeneous canopy shape in *A. viscosus* compared with the shape of *P. canariensis* would explain the higher variability of the labile organic-N fractions found in the legume than in the pine plots.

As we stated in the methods section, we selected isolated individuals that were as close as possible to our pine forest plots in order to maximize the likelihood that the isolation was due to dispersion and colonization processes rather than to pre-existing soil factors. However, this proximity to the pine forest does not eliminate the possibility that the pre-existing conditions were more important than dispersal or colonization processes. If

pre-existing soil factors played an important role in the isolation of the trees studied, our interpretations of the effect of these isolated individuals on the soil nutrient conditions would become weak. Therefore, any conclusions drawn from this study should be interpreted with caution. The limitation of our experimental design in considering pre-existing conditions is a common limitation to many observational studies (Tilman 1989), and it should be taken into account in future research. Long-term manipulative or observational studies, started prior to the establishment of the individual trees, in which soil resources are monitored until the individuals reached the adult phase could help to address this uncertainty.

The results of this study suggest that both the morphological and physiological characteristics of *P. canariensis* and *A. viscosus*, as well as the spatial pattern of *P. canariensis* in an aggregated (pine forest) or a more dispersed (isolated individuals) distribution, may influence the spatial pattern and variability of soil resources. Thus, we could find islands of concentrated nitrate and phosphorus below the understory legumes, whereas the highest concentrations of microbial biomass, dissolved organic-N or ammonium could be found under the pine canopy. Also, the soil spatial variability may be higher under isolated pines than under the pine forest canopy. Finally, our results are in agreement with Binkley et al. (1992), who suggested that the effect of the N₂-fixers is to accelerate N cycling rather than increase its capital.

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