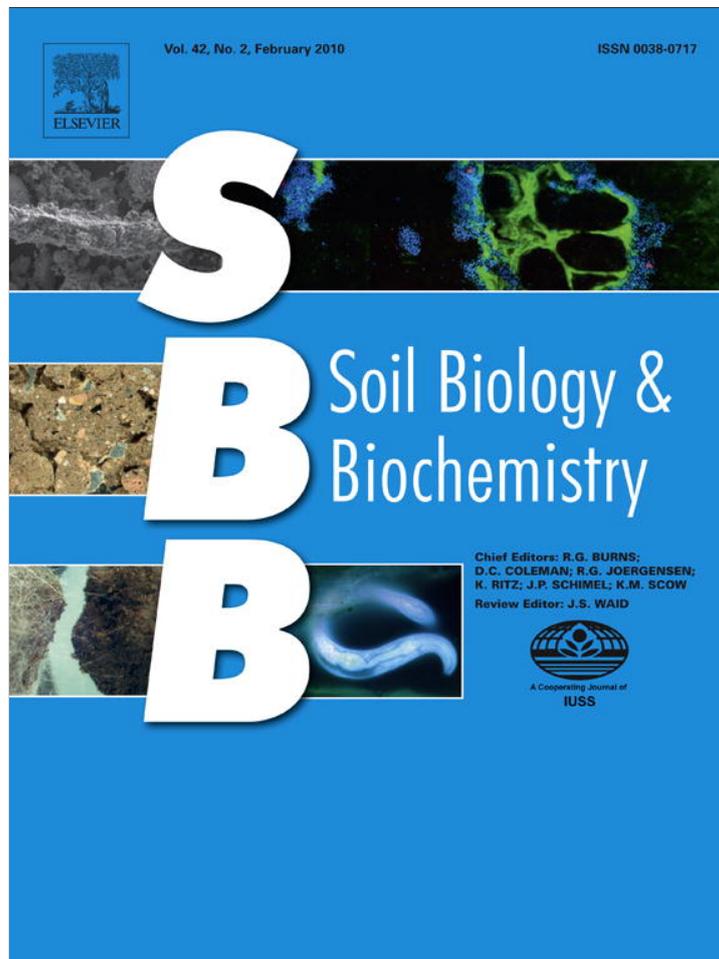


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Short communication

Plants and biological soil crusts modulate the dominance of N forms in a semi-arid grassland

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ABSTRACT

It has been suggested that the dominance of N forms should shift from dissolved organic nitrogen (DON) to nitrate along a gradient of increasing N availability. We aimed to apply this model at a local scale within a semi-arid ecosystem showing a high spatial heterogeneity in the distribution of vegetation and soil resources. By doing this, we seek a better understanding of the N cycling in spatially heterogeneous ecosystems. We took soil samples from the three major sources of spatial heterogeneity: the grass *Stipa tenacissima*, the N-fixing shrub *Retama sphaerocarpa*, and open areas. We also sampled the biological soil crust (BSC) located in the latter areas as another source of spatial heterogeneity. BSC microsites were classified by four levels of soil coverage, ranging from high coverage (66%) to bare soil. The proportion of nitrate, ammonium and DON was determined in all microsites. DON was the dominant N form for open areas, while nitrate was dominant under the canopy of *Retama*; these microsites contained the lowest and highest N availability, respectively. Under BSC, DON was the dominant N form. We found high temporal variability in the dominance of N forms for all microsites. Our results suggest that the biome-derived model of Schimel and Bennett (2004) explaining N form dominance across N availability gradients may be extended to local gradients.

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Grasslands dominated by the tussock grass *Stipa tenacissima* L. are one of the most widespread semi-arid ecosystems in the Mediterranean basin. These ecosystems are highly heterogeneous; they have sparse plant coverage, and biological soil crust (BSC) communities frequently occupy the open spaces located between plant canopies (Maestre and Cortina 2002, Maestre et al. 2009). Schimel and Bennett (2004) suggested a shifting dominance of N forms along a gradient of N availability. Their model focused on differences between large ecosystems types (i.e. boreal forest or tundra vs. agricultural or tropical forest). This study attempts to test whether this model can be applied to spatially heterogeneous ecosystems showing contrasting levels of N availability between plant patches, BSC and adjacent bare ground areas. This approach may allow us to understand whether mechanisms controlling nutrient availability at the biome scale may be also operating at local scales, improving our understanding of the N cycle. Our goal was to analyze the influence of plant coverage and BSC on soil N availability and on the dominance of different inorganic and organic N forms.

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This study was conducted in the Aranjuez experimental station, in the center of the Iberian Peninsula (40°02' N–3°37' W). The climate is Mediterranean semi-arid, with average annual rainfall and temperature of 456 mm and 14 °C, respectively. Perennial plant coverage is lower than 40%, and is dominated by *Stipa* and isolated individuals of the N-fixing shrub *Retama sphaerocarpa* L. Open areas between plant patches contain a well-developed BSC community dominated by lichens such as *Diploschistes diacapsis*, *Squammarina lentigera*, *Fulgensia subbracteata* and *Psora decipiens*. The soil is classified as Xeric Haplogypsid (FAO-ISRIC-ISSS, 1998), and has a fine texture dominated by the presence of gypsum. For the top 10-cm of the soil profile, pH ranged between 7.2 and 7.7; soil C between 1% and 3.2%; and soil N between 0.16% and 0.4%; both extremes corresponding to bare soil and under *Retama* canopies respectively. Two independent samplings were performed. In the first, we identified three sources of spatial heterogeneity: *Stipa* and *Retama* canopies and open areas (irrespective of BSC cover). Five soil samples were collected for each microsite from the top 10 cm of the soil profile. Sampling was carried out with seasonal periodicity during 2007 and 2008. Secondly, we focused on the BSC located in open areas as another source of spatial heterogeneity. Subsequently, for each 2008 sampling event, we collected 5 soil samples from the top 4 cm

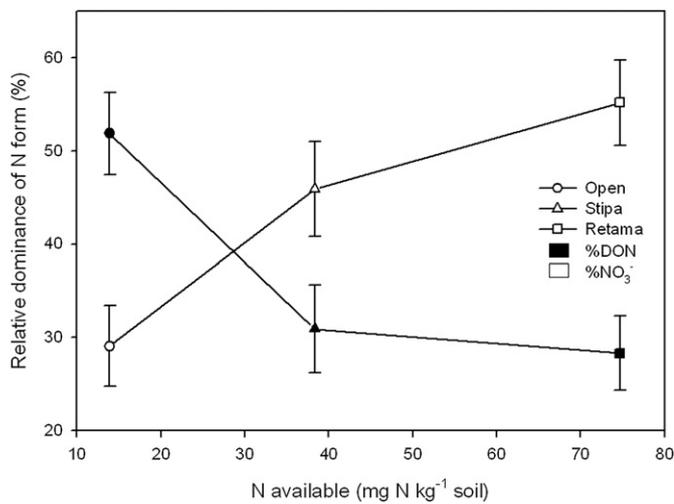


Fig. 1. Relative dominance of $\text{NO}_3\text{-N}$ and DON as a function of local N availability. Stipa, Retama and Open show data collected under the canopy of *Stipa tenacissima* and *Retama sphaerocarpa* and in open areas devoid of vascular plants, respectively. Data represents means \pm SE ($n = 40$).

under each of four microsites: bare soil, and low (below 33%), medium (33–66%) and high (above 66%) BSC cover. Soil samples were transported in coolers and were stored after field collection at 4 °C and processed within 24 h. In the lab, samples were extracted with 0.5 M K_2SO_4 in a ratio 1:5. Soil extracts were shaken in an orbital shaker at 200 rpm for 1 h at 20 °C and filtered to pass a 0.45- μm Millipore filter (Jones and Willett, 2006). The filtered extract was kept at 2 °C until colorimetric analyses, which were conducted within the 24 h following the extraction. Sub-samples of each extract were taken for measurements of Ammonium ($\text{NH}_4^+\text{-N}$), Nitrate ($\text{NO}_3\text{-N}$) and DON concentrations. Ammonium concentration was directly estimated by the indophenol blue method using a microplate reader (Sims et al., 1995). Nitrate was first reduced to NH_4^+ with Devarda alloy and its concentration determined as above. DON in the extracts was first oxidized to NO_3^- with $\text{K}_2\text{S}_2\text{O}_8$ in an autoclave at 121 °C for 55 min, and then reduced to NH_4^+ with Devarda alloy (Sollins et al., 1999).

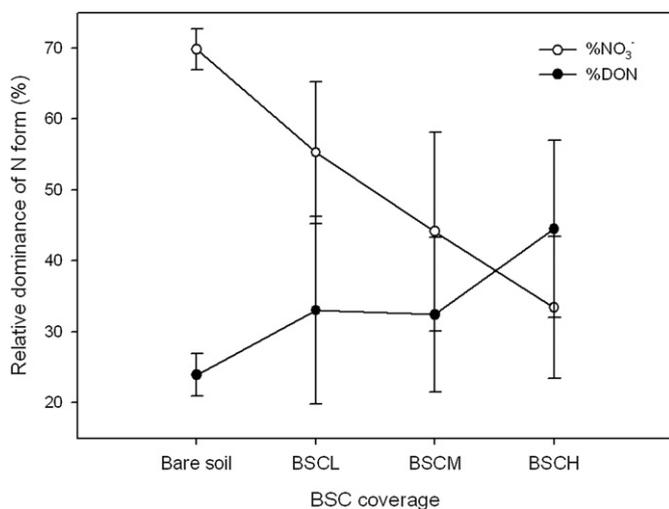


Fig. 2. Relative dominance of $\text{NO}_3\text{-N}$ and DON as a function of biological soil crust (BSC) cover. BSCL, BSCM and BSCH indicate sites with <33%, 33% to 66%, and >66% of BSC cover, respectively. Data represents means \pm SE ($n = 20$).

DON values were calculated as total dissolved N minus inorganic N. The percentage of relative N availability was calculated as the proportion of DON, $\text{NH}_4^+\text{-N}$ or $\text{NO}_3\text{-N}$ relative to total K_2SO_4 -extractable N availability (Schimel and Bennett 2004). All results were calculated on a dry soil basis. Differences in percentage of the soil variables between microsites and sampling dates were tested by using a two-way permutation test (Basso et al., 2009). All statistical analyses were done with the R statistical software, version 2.9.0 (<http://cran.r-project.org/>).

K_2SO_4 -extractable available N (available N) was maximum under the canopy of *Retama* (74.3 mg N kg^{-1} soil), corresponding to the maximum $\text{NO}_3\text{-N}$ proportion found there (Fig. 1). The minimum mean value for N availability was found for the open areas (13.9 mg N kg^{-1} soil), coinciding with the maximum DON

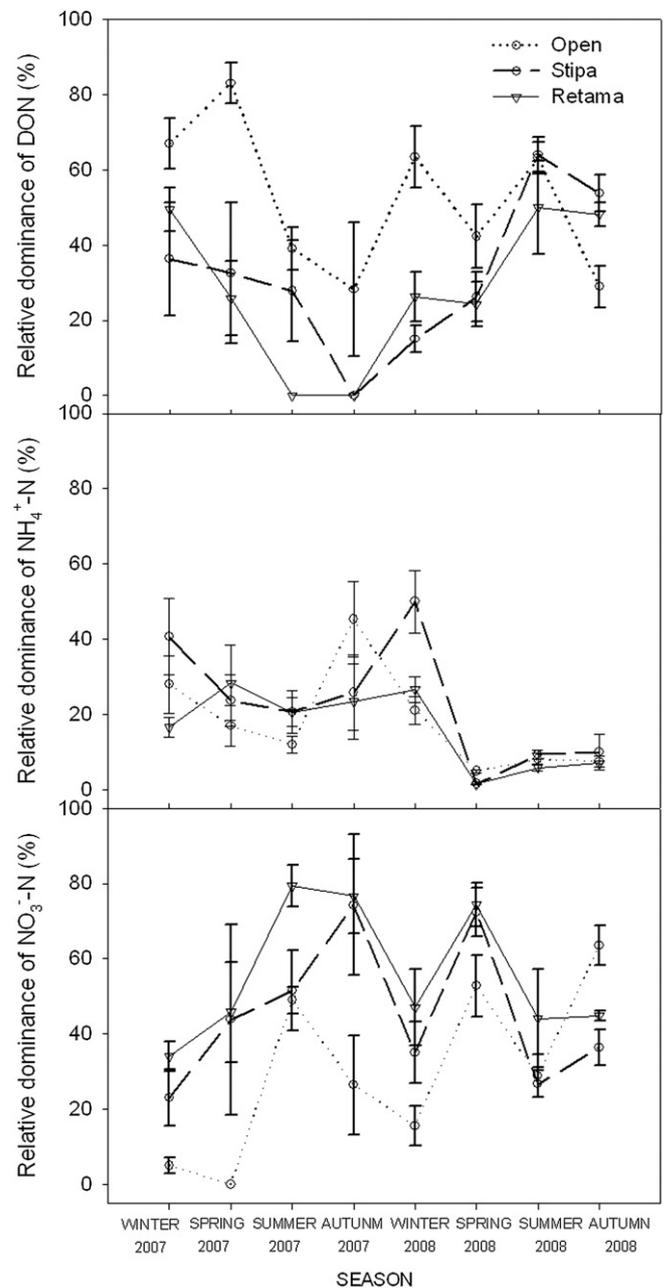


Fig. 3. Seasonal changes in the relative dominance of $\text{NO}_3\text{-N}$, $\text{NH}_4^+\text{-N}$ and DON. Data represents means \pm SE ($n = 5$). Rest of legend as in Fig. 1.

dominance (Fig. 1). Significant differences between microsites were found for NO_3^- -N and DON ($p < 0.01$), but not for NH_4^+ -N. These results suggest that the Schimel and Bennett (2004) model may be applied within a heterogeneous ecosystem with contrasted N availability. Significant differences of N availability and N form dominance were also found in areas of different BSC cover ($p < 0.01$). Mean N availability ranged between $51.2 \text{ mg N kg}^{-1}$ soil and $25.5 \text{ mg N kg}^{-1}$ soil for areas with high and low BSC cover, respectively. NO_3^- -N was the dominant N form for bare soil and low BSC cover areas (Fig. 2), but DON was the dominant N form when the BSC cover was high (Fig. 2). Thus, the cover of BSC in open sites influenced the overall N form dominance, but showed an inverse pattern from that described for

plant microsites. Our results suggest that the biome-scale model developed by Schimel and Bennett (2004) holds in heterogeneous landscapes, where large differences in plant cover exist but it may not hold at finer scales, such as those observed with the BSC portion of this study. The inverse relationship of BSC coverage with NO_3^- -N soil concentration also suggests that BSC may inhibit nitrification. The production of allelopathic and secondary substances by BSC-forming organisms may play a significant role in this inhibition (Fahsel, 1994), albeit further research is needed to confirm this.

Seasonal changes in N form dominance were found for all the microsites evaluated (Figs. 3 and 4). However, the overall tendency described above can be observed throughout all seasons. The observed temporal variability was similar under the canopy of *Stipa* and *Retama*, but that found in the different BSC microsites was remarkably higher than the variability detected in the bare soil (Fig. 4). This suggests a strong seasonal influence of BSC on N dynamics. Differences in seasonal temperature and soil water content may explain changes in dominance of N forms. Thus, the highest DON proportion and the lowest NO_3^- -N proportion found for 2008 coincided with the lowest mean temperature (6.6°C) and soil water content (0.6–6%) found during the sampling interval for all microsites.

Our results show that the Bennett's model is valid for comparisons between microsites with differing N availability within a semi-arid heterogeneous ecosystem. However, the dominance of N forms is also modulated by intra- and inter-annual climatic variability and by the presence of the BSC, which causes a pattern on N form dominance inverse to that predicted by this model. The application of the biome-derived model to the local-scale might be appropriate in semiarid ecosystems where contrasting N availability correlates with the spatial heterogeneity in plant cover.

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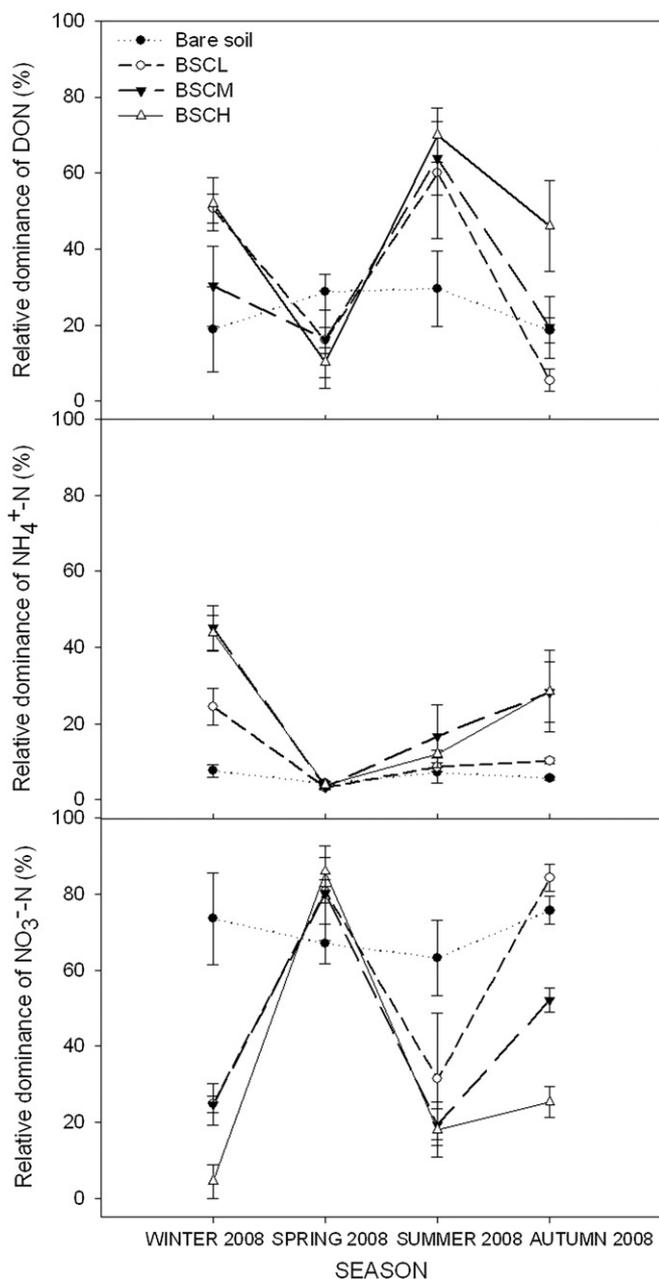


Fig. 4. Seasonal changes in the relative dominance of NO_3^- -N, NH_4^+ -N and DON at open microsites with contrasting BSC cover. Data represents means \pm SE ($n = 5$). Rest of legend as in Fig. 2.