Are phenolic compounds released from the Mediterranean shrub *Cistus albidus* responsible for changes in N cycling in siliceous and calcareous soils?

Eva Castells, Josep Peñuelas and David W Valentine

Summary

- We studied the effects of *Cistus albidus* leaf leachates on nitrogen-cycling processes in two siliceous soils (granite and schist) and one calcareous soil. We compared those effects with gross N-transformation rates in soils sampled underneath *Cistus*.

- Soils amended with leachates and soils sampled under *Cistus* had higher NH$_4^+$ immobilization and lower nitrification compared with control soils. Gross N mineralization increased under *Cistus* but decreased in soils amended with leachates. These effects were especially strong in granite soil.

- To determine whether phenolic compounds were causing those effects, we incubated granite soils with leachate and a leachate fraction containing only nonphenolic compounds. Nonphenolic compounds increased NH$_4^+$ immobilization and decreased gross nitrification, while decreases in gross N mineralization were estimated to be caused by phenolic compounds.

- Our results show that although phenolic compounds leached from green foliage changed gross N mineralization, their effects on net N rates were eclipsed by the changes produced by polar nonphenolic compounds such as carbohydrates. Plant non phenolic compounds may drive N cycling under *Cistus*.

Key words: phenolics, N cycling, $^{15}$N isotope dilution, N immobilization, Mediterranean vegetation, siliceous soils, calcareous soils, *Cistus albidus*.


Introduction

Phenolic compounds are a widely distributed group of plant carbon-based secondary metabolites which have been related to various ecological functions such as plant-herbivore interactions and pollination (Waterman & Mole, 1994). Phenolic compounds may also play a role in controlling many aspects of plant-soil interactions, including regulation of nutrient cycling and organic matter dynamics, and alteration of soil nutrient availability by either increasing or decreasing microbial activity (Kuiters, 1990; Schimel et al., 1996; Northup et al., 1998). Phenolic compounds have been shown to decrease soil N availability by (i) forming complexes with proteins, thus delaying organic matter decomposition and mineralization (Horner et al., 1988; Nicolai, 1988; Palm & Sanchez, 1990; Harrenschwiler & Vitousek, 2000); (ii) increasing microbial activity and nitrogen immobilization (Sparling et al., 1981; Blum & Shafer, 1988; Shafer & Blum, 1991; Sugai & Schimel, 1993; Schimel et al., 1996; Blum, 1998); (iii) inhibiting nitrification (Baldwin et al., 1983; Rice, 1984); and (iv) inhibiting fungal respiration (Boufalis & Pellissier, 1994). The nature of the phenolic compounds has been related to the different effects on N cycling. Thus the high molecular-weight condensed tannins were more involved in linking organic matter and slowing decomposition, while low molecular-weight phenolics were more easily degraded by microorganisms when used as a C source (Fierer et al., 2001). As concentration and type of phenolic compounds are strongly determined by plant
specific bedrock types: granodioritic (here termed granitic, for simplicity); schistic; and calcareous, which differ in their physical and chemical properties (Table 1). The mean annual precipitation of this region is about 614 mm and the mean annual temperature is 13.9°C. The sampling areas for each bedrock type were established within 600 m of one another at the same elevation (ca. 800 m) on south or south-west aspects to minimize differences in temperature and precipitation. The plant community in the three bedrock types was a low, open shrubland dominated by Cistus albidus (L.) and Quercus ilex (L.). More information on vegetation cover on each of the bedrock types is given by Castells & Peñuelas (2003).

*Cistus albidus* (hereafter termed *Cistus*), a Mediterranean evergreen shrub with leaf longevity not longer than 1 yr found in either siliceous (granite or schist) or calcareous soils, was selected for this study because of its capacity for leaching high concentrations of phenolic compounds from green leaves compared with other Mediterranean shrubland species from the same plant community (Table 2). The mean annual precipitation of this region is about 614 mm and the mean annual temperature is 13.9°C. The sampling areas for each bedrock type were established within 600 m of one another at the same elevation (ca. 800 m) on south or south-west aspects to minimize differences in temperature and precipitation. The plant community in the three bedrock types was a low, open shrubland dominated by *Cistus albidus* (L.) and *Quercus ilex* (L.). More information on vegetation cover on each of the bedrock types is given by Castells & Peñuelas (2003).

The importance of plant phenolic compounds released from foliage and litter on soil N-cycling rates is mostly unknown. The effects of phenolics on N cycling have traditionally been tested for by adding to the soil a single phenolic or a mixture of phenolic compounds either of synthetic origin or purified from plant tissue (Sparling et al., 1981; Blum & Shafer, 1988; Shafer & Blum, 1991; Sugai & Schimel, 1993; Boufalis & Pellissier, 1994; Schimel et al., 1996; Inderjit & Mallik 1997; Blum, 1998; Bradley et al., 2000; Magill & Aber, 2000; Fierer et al., 2001). Several problems arise when using this approach to determine the role of phenolics under natural conditions. On one hand, phenolic compounds leached from the plant foliage and leaf litter span a range of molecular weights and have different abilities to interfere with N cycling (Hattenschwiler & Viroušek, 2000; Fierer et al., 2001), and the effects of a single phenolic compound or a partial mixture may not account for the overall effects of the phenolics released to the soil. Moreover, the effects of a phenolic compound mixture have been shown to be stronger than the effects of single compounds in some cases (Inderjit & Mallik, 1997). In order to test the effects of a phenolic compound mixture closer to natural conditions, we obtained a foliage leachate from a phenolic-rich species, the Mediterranean shrub *Cistus albidus*, and studied the effects of the leachate and the leachate non-phenolic fraction on soil net and gross N transformation processes. We were therefore able to quantify the relative importance of the phenolic compounds compared with other soluble C compounds present in the leachates that could also affect soil N cycling. In order to assess whether the leached C compounds, including phenolic compounds, could change soil processes similarly to the effects of the plant, and whether soil properties could influence those changes, we compared the effects of the whole leachate and its fractions with the effects of plant canopy presence on soil N cycling in three soils (two siliceous and one calcareous soil) with different physical and chemical properties.

### Materials and Methods

**Sites and plant description**

The study sites were located in Capafons (Prades Mountains, south-west of Barcelona, Spain) on soils derived from three different bedrock types: granodioritic (here termed granitic, for simplicity); schistic; and calcareous, which differ in their physical and chemical properties (Table 1). The mean annual precipitation of this region is about 614 mm and the mean annual temperature is 13.9°C. The sampling areas for each bedrock type were established within 600 m of one another at the same elevation (ca. 800 m) on south or south-west aspects to minimize differences in temperature and precipitation. The plant community in the three bedrock types was a low, open shrubland dominated by *Cistus albidus* (L.) and *Quercus ilex* (L.). More information on vegetation cover on each of the bedrock types is given by Castells & Peñuelas (2003).

**Table 1** Physical and chemical properties of soils on study sites (Castells & Peñuelas, 2003)

<table>
<thead>
<tr>
<th>Property</th>
<th>Granitic</th>
<th>Schistic</th>
<th>Calcareous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of soil (cm)</td>
<td>0–20</td>
<td>0–19</td>
<td>0–17</td>
</tr>
<tr>
<td>Horizons sampled</td>
<td>A, 8</td>
<td>A, 8</td>
<td>A1</td>
</tr>
<tr>
<td>pH</td>
<td>7.1</td>
<td>6.3</td>
<td>7.8</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>0.06</td>
<td>0.12</td>
<td>0.28</td>
</tr>
<tr>
<td>(dS m⁻¹) (1:5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCO₃ (%)</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>1.4</td>
<td>2.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Organic N (N Kjeldahl)</td>
<td>0.1</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td>Phosphorus [P] (mg kg⁻¹)</td>
<td>3</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Potassium [K⁺] (mg kg⁻¹)</td>
<td>87</td>
<td>398</td>
<td>273</td>
</tr>
<tr>
<td>Sand (% of fine earth)</td>
<td>93.8</td>
<td>58.3</td>
<td>36.9</td>
</tr>
<tr>
<td>Silt (% of fine earth)</td>
<td>3.9</td>
<td>31.8</td>
<td>38.9</td>
</tr>
<tr>
<td>Clay (% of fine earth)</td>
<td>2.2</td>
<td>9.9</td>
<td>24.2</td>
</tr>
<tr>
<td>USDA classification</td>
<td>sand</td>
<td>sandy loam</td>
<td>loam</td>
</tr>
</tbody>
</table>

**Table 2** Single determinations of total phenolic compounds of green leaves and shoot leachates from six species commonly present in the Mediterranean

<table>
<thead>
<tr>
<th>Species</th>
<th>Gallic acid equivalent (mg g⁻¹ d. wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercus cocciifera (L.)</td>
<td>0.39</td>
</tr>
<tr>
<td>Quercus ilex (L.)</td>
<td>0.35</td>
</tr>
<tr>
<td>Cistus albidus (L.)</td>
<td>1.73</td>
</tr>
<tr>
<td>Cistus monspeliensis (L.)</td>
<td>0.88</td>
</tr>
<tr>
<td>Erica arborea (L.)</td>
<td>0.24</td>
</tr>
<tr>
<td>Erica multiflora (L.)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Plants were sampled during January 2000; leachates were made as described in Materials and methods.
studied, *Cistus* cover ranged from 20 to 30%. Although shoot biomass of *Cistus* was larger in granite and schist soils, no differences in canopy density were found among plants growing at different bedrock types (Castells & Penuelas, 2003).

**Soil sampling**

Four sites within an average of 75 m from one another were located during July 2000 at each bedrock type (12 sites in total). At each site we established five treatment plots that included a *Cistus* plant, and then established five adjacent control plots without *Cistus* and within 3 m of each treatment plot. The control plots were along the same elevation contour as, or slightly upslope of, the adjacent treatment plots to avoid the effect of leaf or litter leachates. Control plots at the granitic and calcareous sites were not covered by vegetation, while herbaceous vegetation was present at the schistic sites. Soils from *Cistus* plots were taken under a plant individual, where litter was present, preferentially on the lower side of the slope.

The top 15 cm of mineral soil was sampled using a 5 cm diameter corer. One soil core from control plots and one core underneath the *Cistus* canopy in treatment plots were sampled (five control samples and five treatment samples per site and bedrock type), and bulked together within treatment for each of the sites. Soils were carried to the laboratory, sieved with a 2 mm mesh and kept at 4°C for a maximum of 1 wk before being analysed.

**Leachate preparation and fractionation**

A preliminary assay was conducted of total phenolic compounds in separated leachates made with green leaves and with standing leaf litter, collected in July 2000, to determine which yielded the greatest amount of phenolic compounds. Although the leaching of phenolics from the litter layer by rainfall has been shown to be higher than from green leaves for some species (Kuiters, 1990), our results on standing litter and leaves showed a larger concentration in the latter (14.3 mg gallic acid g\(^{-1}\), d. wt, vs 20 mg gallic acid g\(^{-1}\), d. wt). Additionally, as the release of phenolic compounds from green foliage in evergreen species such as *Cistus* is expected to be less variable with time than release from decomposing litter, green leaf leachates were selected when conducting the soil incubation experiments and leachate fractionation.

A *Cistus* leachate was obtained by shaking fresh green leaves (25 g, d. wt equivalent) collected from plants growing in a schist-derived soil in 11 distilled water for 24 h at room temperature after filling the headspace with \(N_2\) (Zackrisson & Nilsson, 1992). The resulting leachate was filtered through Whatman no. 42 filter paper and kept at -20°C. The same leachate stock was used in all experiments.

The leachate was fractionated into phenolic and non-phenolic fractions by solid-phase extraction using C18 Extra-sep columns (Lida Manufacturing Corp, Kenosha, WI, USA) which retain phenolic compounds. After conditioning the columns with methanol, filtered leachate was passed through the column and the polar substances (nonphenolic fraction) were collected. Intact leachate (hereafter termed leachate), the nonphenolic fraction and a blank of distilled water (control) were kept at 4°C and used the following day to amend soils. The nonpolar fraction containing phenolic compounds was eluted from the column with methanol. The leachate and nonphenolic and phenolic fractions were analysed for total phenolics to confirm the efficiency of the column. Total phenolic compound concentrations, including condensed tannins, were determined using a modified Folin-Ciocalteu method in which a blank of polyvinylpolypyrrolidone (PVPP) (Marigo, 1973) was used. PVPP removes phenolic substances from the solution, avoiding an overestimation of total phenolics because of nonphenolic Folin-Ciocalteu-reactive substances. Gallic acid was used as a standard. Condensed tannins in leachate were analysed using the proanthocyanidin method (Waterman & Mole, 1994) with cyanidine chloride as a standard. Dissolved organic carbon (DOC) in the leachate and in the nonphenolic fraction was analysed using a Shimadzu TOC-5000 analyser (Shimadzu Corporation, Kyoto, Japan). After analysis the phenolic fraction was discarded, and only the intact leachate and the nonphenolic fraction were retained for use in experiments.

**Gross N-transformation rates in soils under *Cistus***

Gross rates of N mineralization (ammonification), \(NH_4^+\) consumption, gross nitrification and \(NO_3^-\) consumption were determined by \(^{15}\)N isotope dilution (Hart et al., 1994a) in soils sampled under *Cistus* and in control plots (four replicates, one for each site, per *Cistus* treatment and bedrock type). Soils under *Cistus* were amended with distilled water; control soils were amended with *Cistus* leachate or distilled water (control). A solution containing 3.5 ml leachate or distilled water and 1.5 ml \(^{15}\)NH\(_4^+\) or \(^{15}\)NO\(_3^-\) (0.05 mg \(^{15}\)N, 99 at. %) was added to 40 g soil placed in plastic cups of approx. 5 cm diameter. The cups that received \(^{15}\)NH\(_4^+\) were used to estimate gross N mineralization and \(NH_4^+\) consumption, and the cups that received \(^{15}\)NO\(_3^-\) were used to estimate gross nitrification and \(NO_3^-\) consumption. Additional distilled water was added to the soils until field capacity, which was measured on a soil subsample per site and bedrock type using a modified procedure from Tan (1996). To distribute the \(^{15}\)N homogeneously within each cup, small volumes of the isotope solutions were injected multiple (10-12) times into each soil sample until all the solution was applied. Two cups per soil sample were injected. Soil from one cup was homogenized for 3 min, and 15 g soil were immediately extracted for 1 h with 75 ml 2 M KCl. The other cup was incubated at room temperature and extracted after approx. 24 h, and incubation time was recorded. Initial and final KCl extracts were analysed for \(NH_4^+\) or \(NO_3^-\) concentrations (from the soils that received \(^{15}\)NH\(_4^+\) or \(^{15}\)NO\(_3^-\), respectively) using an auto-analyser (Flow Injection...
Analyses, FOSS, Hoganas, Sweden). For the samples that received $^{15}$NH$_4$+, the KCl extracts were basified by adding MgO, and the NH$_3$ vapour was released and captured on an acidified disk of filter paper (Whatman no. 5), after Holmes et al. (1998). For the samples that received $^{15}$NO$_3$ - we allowed the NH$_3$ to escape for 6 d, and Devardas alloy was added to reduce the NO$_3$- to NH$_4$+. The NH$_3$ was captured by a filter-paper disk as described above. Filter papers were analysed for $^{15}$N at. % using an isotope ratio mass spectrometer (20-20 PDZ Europa, Cheshire, UK). Gross N-transformation rates were calculated from changes in NH$_4$ + or NO$_3$ - concentrations and changes in $^{15}$N at. % during the incubation following the equations derived by Kirkham & Bartholomew (1954). We assumed the background $^{15}$N enrichments to be 0.37% at. % $^{15}$N (Hart et al., 1994a). NH$_4$+ consumption rates obtained from Kirkham & Bartholomew’s (1954) equations were used to calculate NH$_4$ + immobilization rates by subtracting gross N nitrification from NH$_4$ + consumption rates. NO$_3$ - consumption rates were assumed to be caused entirely by NO$_3$ - immobilization. Rates were expressed per unit organic C. Although isotope addition may overestimate NH$_4$+ and NO$_3$ - immobilization rates (Hart et al., 1994a), this bias was expected to be similar among Cistus treatments and bedrock types because the same amount of $^{15}$N was added to each sample, and thus should not interfere when comparing the response of different soils. Moreover, the addition of $^{15}$N does not always result in a stimulation of N immobilization ambient rates, and strong significant correlations have been found between N-immobilization rates estimated using the $^{15}$N pool dilution method and rates estimated in samples that did not receive $^{15}$N amendment (Hart et al., 1994b).

Net and gross N-transformation rates after addition of fractionated leachate

We estimated net N mineralization and soil respiration in soils from control plots sampled at granite, schist and calcareous bedrock types, and amended with distilled water (control), leachate or the nonphenolic fraction of the leachate. Because we were interested in comparing the effects of the leachate and the nonphenolic fraction, soils from different sites were bulked together within bedrock type to obtain homogeneous samples. Three replicates of the bulk soil (30 g, f. wt) per bedrock type were placed in a 425 ml glass jars with a septum that allowed air samples to be taken. Soils were amended with 3.6 ml distilled water, leachate or nonphenolic fraction, and incubated at 25°C. Additional distilled water was added to the soils until field capacity was reached, when necessary. A soil subsample (15 g, f. wt) was extracted with 75 ml KCl for 1 h at the onset of incubation, and analysed for initial NH$_4$+ and NO$_3$ - using an auto-analyser (Flow Injection Analyses). Carbon mineralization was estimated during the entire incubation by analysing, every 4-7 d, the CO$_2$ accumulated in jars using a gas chromatograph with a Porapaq QS column and a thermal conductivity detector (Hewlett Packard 5890 Series II). After measurements, jars were opened and ventilated to re-establish ambient CO$_2$ concentrations, and distilled water was added to maintain field capacity when necessary. After 28 d, soils were analysed for final NH$_4$+ and NO$_3$ - concentrations. Net N-mineralization rates were calculated by subtracting initial from final concentrations, expressed per unit organic C. Organic C was analysed using an elemental analyser (model NA 1500, Carlo Erba, Milan, Italy). Carbon mineralization was calculated from total CO$_2$ produced during each measurement period in the incubation. We calculated the ratio N : C mineralization by dividing net N mineralization by C mineralization for the entire incubation.

Gross rates of N mineralization, NH$_4$+ consumption, gross nitrification and NO$_3$ - consumption were determined by $^{15}$N isotope dilution in granite-derived soils not associated with Cistus, and incubated with distilled water (control), leachate and the nonphenolic fraction.

Statistical analyses

We conducted a two-way ANOVA to test for the effects of Cistus presence and leachate addition on gross N-transformation rates in granite-, schist- and calcareous-derived soils. A two-way ANOVA with bedrock type and treatment (soils under Cistus or leachate addition) as main effects was performed instead of a three-way factorial ANOVA with canopy presence and leachate addition (Castells & Penuelas, 2003). A two-way ANOVA was also conducted to test the effect of leachate fractions on net N-mineralization rates and N : C mineralization. A two-way repeated-measure ANOVA with bedrock type and treatment as independent variables, and date of sampling as a repeated variable, was conducted to test for changes in C mineralization rate. Finally, a t-test was conducted to evaluate the effects of leachate fractions on gross N-transformation rates. We also performed post hoc comparisons using Tukey’s HSD test ($P < 0.05$) to compare the effects of leachate fractions on net and gross transformation rates. No transformations were required to fit a normal distribution. All analyses were performed using STATISTICA 99 (Statsoft, Inc., Tulsa, OK, USA).

Results

Leachate fraction analyses

Leachate contained 330 mg 1-1 total phenolics, 67 mg 1-1 condensed tannins, and 1191 mg 1-1 DOC. The leachate nonphenolic fraction contained 38 mg 1-1 total phenolics and 640 mg 1-1 DOC. Retention of phenolic compounds by the
Effects of leachate fractions on N transformations

Net N mineralization decreased by 9% compared with the controls in granite-, schist- and calcareous-derived soils when amended with the nonphenolic fraction, while C mineralization increased by 7% (Fig. 1; Table 3). The addition of the nonphenolic fraction accounted for 56 and 54% of the leachate effects on net N mineralization and N : C mineralization, respectively, when averaged for all bedrock types. Although the increase in C mineralization was statistically significant for all soils and dates of sampling, a substantial effect was found only in granite soil in the first week of incubation (Fig. 2; Table 3). The N : C mineralization ratio, which is an indicator of the relative limitation of C or N, was lower in soils amended with nonphenolic fraction compared with the control (-15%). Bedrock type had no effect on the response of net N mineralization and N : C mineralization to treatment amendment (Fig. 1; Table 3).

Granite soils showed a significant increase in NH$_4^+$ immobilization rates (9.7 and 8.5 μg N g$^{-1}$ C d$^{-1}$ respectively) compared with the control when soils were amended with the non phenolic fraction or intact leachate. The addition of leachate also increased NO$_3^-$ immobilization rates (7.3 μg N g$^{-1}$ C d$^{-1}$). The nonphenolic fraction and the leachate marginally decreased gross N nitrification rates (4.5 μg N g$^{-1}$ C d$^{-1}$) and gross N mineralization (7.1 μg N g$^{-1}$ C d$^{-1}$), respectively (Fig. 3). Non-significant differences in post hoc comparisons between the non phenolic fraction and the leachate indicated that changes in NH$_4^+$ immobilization after leachate addition could be attributed entirely to the effects of the nonphenolic compounds (data not shown).

### Table 3 Statistical analyses for net N mineralization, CO$_2$ production (C mineralization) and ratio between N mineralization and C mineralization in granite, schist and calcareous substrates (Bedrock) amended with distilled water, Cistus leachate or nonphenolic fraction of Cistus leachate (Treatment)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Net N mineralization</th>
<th>C mineralization</th>
<th>N : C mineralization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Bedrock</td>
<td>2</td>
<td>825.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>15.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>2.67</td>
<td>0.065</td>
</tr>
<tr>
<td>Bedrock × Treatment</td>
<td>4</td>
<td>366.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control – Leachate</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control – Nonphenolics</td>
<td>-</td>
<td>0.018</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leachate – Nonphenolics</td>
<td>-</td>
<td>0.055</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

A two-way ANOVA was conducted for net N mineralization and N : C mineralization ratio. A repeated-measures ANOVA (Time) was conducted for C mineralization. Post hoc comparisons for Treatment were done using a Tukey's HSD test. $n = 3$. P < 0.05 in bold type.
contrasting effects of plant presence and leachate addition (Fig. 4). NH$_4^+$ immobilization rates under Cistus and after leachate addition increased by 50 and 41%, respectively, compared with controls, while gross N nitrification rates decreased by 54 and 41% (Fig. 4). Changes in NH$_4^+$ immobilization and gross N nitrification were greatest in granitic soils, although only a marginally significant interaction between bedrock type and treatment was found in both dependent variables ($P = 0.06$, ANOVA).

Fig. 2 Soil respiration along a 4 wk incubation of granite, schist and calcarious soils amended with distilled water (control), nonphenolic fraction of Cistus leachates, and Cistus leachates. Each value represents the means ± SE ($n = 3$) for the CO$_2$ emitted during the time interval since the previous sampling. Statistical analyses are shown in Table 3.

Fig. 3 Gross N transformation processes of granite soils amended with distilled water (control), nonphenolic fraction of Cistus leachates, and Cistus leachates. Values represent means ± SE ($n = 4$). Post hoc comparisons between control and nonphenolics, and control and leachate are shown.
conditions of moisture, temperature and soil chemical and physical properties, NH$_4^+$ immobilization can increase because of labile C compounds released from canopy, such as carbohydrates and phenolic acids, when microbes used them as a substrate (Sparling et al., 1981; Blum & Shafer, 1988; Shafer & Blum, 1991; Sugai & Schimel, 1993; Schimel et al., 1996; Blum, 1998), and/or by chemical immobilization by organic matter (Nommik & Vahtras, 1982). In our study, Cistus leachate and nonphenolic fraction similarly increased NH$_4^+$ immobilization and decreased gross nitrification. Thus phenolic compounds present in leachates had no effect on any of those processes, which were caused by the nonphenolic fraction formed by highly polar compounds such as carbohydrates. Our data therefore suggest that decreases in nitrification were caused directly by decreases in available NH$_4^+$ when microbes used the soluble C compounds as an energy substrate. A similar result was found by Stienstra et al. (1994). The dominance of N immobilization is expected to occur when a limited NH$_4^+$ supply raises competition between microorganisms. Under these conditions, NH$_4^+$-oxidizing bacteria are generally less successful than heterotrophic microorganisms (Riha et al., 1986).

**Discussion**

In a previous study (Castells & Peñuelas, 2003) we found a decrease in net N mineralization after addition of Cistus leachate. Here we aimed to find out what percentage of this effect was caused by nonphenolic compounds present in the leachate. The addition of Cistus leachate, and the nonphenolic fraction of the leachate to granite-, schist- and calcareous-derived soils, decreased net N mineralization and N : C mineralization. Because the effects of the nonphenolic fraction on net N rates accounted for just 56% of the effects of the whole leachate, both phenolic and non-phenolic compounds were responsible for the changes produced on N cycling. We conducted a pool-dilution experiment to find out what particular pathways were affected by both phenolics and nonphenolics. Gross N mineralization in granite tended to decrease in the presence of leachate, but no effect was found in the presence of the non-phenolic fraction, suggesting that changes in gross N mineralization were caused by the phenolic compounds present in the leachate. High molecular-weight condensed tannins from Cistus may play this role, as these compounds have been shown to slow down decomposition and mineralization by forming recalcitrant complexes with organic matter (Hattenschwiler & Vitousek, 2000; Fierer et al., 2001).

Averaged for all soils, the addition of leachate also increased NH$_4^+$ immobilization and decreased gross N nitrification. As both processes share the same substrate (NH$_4^+$), the question arises: was the decrease in nitrification caused by an increase in NH$_4^+$ immobilization, or was nitrification inhibited through toxic effects on nitrifiers, and then immobilization increased because more NH$_4^+$ was available? The competition between ammonifying bacteria and NH$_4^+$-oxidizing bacteria (nitrifiers) has been studied to explain decreases in nitrification along succession (Stienstra et al., 1994). Under similar conditions of moisture, temperature and soil chemical and physical properties, NH$_4^+$ immobilization can increase because of labile C compounds released from the canopy, such as carbohydrates and phenolic acids, when microbes used them as a substrate (Sparling et al., 1981; Blum & Shafer, 1988; Shafer & Blum, 1991; Sugai & Schimel, 1993; Schimel et al., 1996; Blum, 1998), and/or by chemical immobilization by organic matter (Nommik & Vahtras, 1982). In our study, Cistus leachate and nonphenolic fraction similarly increased NH$_4^+$ immobilization and decreased gross nitrification. Thus phenolic compounds present in leachates had no effect on any of those processes, which were caused by the nonphenolic fraction formed by highly polar compounds such as carbohydrates. Our data therefore suggest that decreases in nitrification were caused directly by decreases in available NH$_4^+$ when microbes used the soluble C compounds as an energy substrate. A similar result was found by Stienstra et al. (1994). The dominance of N immobilization is expected to occur when a limited NH$_4^+$ supply raises competition between microorganisms. Under these conditions, NH$_4^+$-oxidizing bacteria are generally less successful than heterotrophic microorganisms (Riha et al., 1986).

Besides incubating soils with Cistus leachates and leachate fractions, we also analysed gross N- transformation rates in soils sampled under Cistus in order to elucidate whether phenolic compounds leached from the canopy could be one of the factors affecting soil N cycling under natural conditions. The estimated effect of phenolics, averaged for all soils, explained 45% of the leaf N effects on net N mineralization. However, phenolics were not quantitatively relevant under Cistus because the addition of leachate decreased gross N mineralization, while soils sampled under the canopy had higher gross N mineralization rates compared with control soils. These results suggest that, although the decrease in gross N mineralization...
caused by phenolic compounds can potentially occur under natural conditions. The release of other compounds from the plant, including above-ground inputs of labile C compounds from leaves and litter and below-ground inputs from root exudation or root decomposition, are likely to have stronger effects on gross N mineralization. Two lines of evidence suggest that labile C compounds would drive \( \text{N} \) cycling under \textit{Cistus}. First, increases in \( \text{NH}_4^+ \) immobilization and decreases in gross \( \text{N} \) nitrification caused by non-phenolic compounds were similar between soils amended with leachate and soils sampled under \textit{Cistus}. Second, higher gross \( \text{N} \) mineralization, as found under \textit{Cistus}, is expected to be coupled with the use of C compounds as a substrate by microbes because C additions increase microbial biomass and N turnover (Clein \\& Schimel, 1995; Bradley et al., 1997). Although microbial biomass was not measured in our experiment, the increases in C mineralization under \textit{Cistus} soils (Castells \\& Penuelas, 2003) suggest that this process was present. These compounds, then, are playing a role in determining \( \text{N} \) cycling in those processes where \( \text{NH}_4^+ \) is a substrate, but not in those related to the generation of \( \text{NH}_4^+ \).

\section*{Conclusions}

Both nonphenolic and phenolic compounds present in \textit{Cistus} leachate changed soil \( \text{N} \) cycling. Although their effects were similar in all bedrock types, their effects were stronger in granite. Our results show that, while phenolic compounds leached from the canopy can change gross \( \text{N} \) mineralization and \( \text{NO}_3^- \) immobilization, these effects do not seem relevant when considering the whole-plant effect on soil. Similar effects of nonphenolic compounds and \textit{Cistus} presence on soil \( \text{N} \) transformations suggest that nonphenolic rather than phenolic compounds are more important in soil \( \text{N} \) cycling.

\section*{Acknowledgements}

We thank Josep Maria Alcaniz, Ramon Vallejo and Arthur Zangerl for critically reading the manuscript. We also thank Marc Estiarte for his assistance during the \( ^{15}\text{N} \) pool dilution experiments and for helpful advice in the laboratory. E.C. received a predoctoral fellowship (FPI) from the Ministerio de Educacion y Cultura (Spain) in collaboration with Carburos Metálicos SA. We thank financial support from CICYT grants REN2000-0003 and REN2001-0278 (Spanish Government).

\section*{References}


Bradley RL, Titus BD, Preston CP. 2000. Changes to mineral \( \text{N} \) cycling and microbial communities in black spruce humus after additions of (\( \text{NH}_4 \))\textsubscript{2}\text{SO}_4 and condensed tannins extracted from \textit{Kalmia angustifolia} and balsam fir. \textit{Soil Biology and Biochemistry} 32: 1227-1240.


New Phytologist

About New Phytologist

- New Phytologist is owned by a non-profit-making charitable trust dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.

- Regular papers, letters, Research reviews, Rapid reports and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication ‘as-ready’ via OnlineEarly - average first decisions are just 5-6 weeks. Essential colour costs are free, and we provide 25 offprints as well as a PDF (i.e. an electronic version) for each article.

- For online summaries and ToC alerts, go to the website and click on ‘Journal online’. You can take out a personal subscription to the journal for a fraction of the institutional price. Rates start at £108 in Europe/$193 in the USA & Canada for the online edition (click on ‘Subscribe’ at the website).

- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 592918) or, for a local contact in North America, the USA Office (newphytol@ornl.gov; tel 865 576 5261).