Capítol 3

3. Carbon-based secondary and structural compounds in Mediterranean shrubs growing near a natural CO_2 spring

J. PEÑUELAS [1], E. CASTELLS [1], R. JOFFRE [2] and R. TOGNETTI [3]

[1] Unitat d'Ecofisiologia CSIC-CREAF, Centre de Recerca Ecològica i Aplicacions Forestals (CREAF), Facultat de Ciències, Universitat Autònoma, Bellaterra 08193 (Barcelona), Spain

[2] DREAM Unit, Centre d'Ecologie Fonctionnelle et Evolutive-CNRS, 1919 route de Mende, 34293 Montpellier Cedex 5, France

[3] Dipartimento di Scienze Animali, Vegetali e dell'Ambiente (SAVA), Universitá degli Studi del Molise, via De Sanctis I-86100 Campobasso, Italy

(acceptat a Global Change Biology, 2001 i pendent de publicació)

3.1. Abstract

We studied carbon-based secondary and structural compounds (CBSSC) in *Myrtus communis, Erica arborea,* and *Juniperus communis* co-occurring in a natural CO_2 spring and in a nearby control site in a Mediterranean environment. Leaf concentrations of phenolics and CBSSC such as lignin, cellulose, and hemicellulose, total non structural carbohydrates (TNC) and lipids were measured monthly (phenolics) and every two months (the other compounds) throughout a year. There was a slight seasonal trend towards maximum concentrations of most of these CBSSC during autumn-winter and minimum values during the spring season, particularly in *Myrtus communis*. For most of the CBSSC and species there were no consistent or significant patterns in response to the elevated [CO_2] (ca. 700 μmol mol^{-1}) of the spring site. These results were not due to a dilution effect by increased structural or non-structural carbon. Therefore, in contrast to many experimental studies of CO_2 enrichment, mainly conducted for short-periods, there were no greater concentrations of phenolics, and, as in many of these studies, there were neither greater concentrations of the other CBSSC. These results do not agree with the predictions of the carbon source-sink hypotheses. Possible causes of this disagreement are discussed. These causes include the complex heterogeneous environmental conditions and the variability of resource availabilities in the field, photosynthetic down regulation, and/or the homeostatic and evolutionary nature of organisms. These results suggest evolutionary adaptive responses to changes in CO_2. They also suggest caution in attributing increased CBSSC concentrations to elevated [CO_2] at long-term scale in natural conditions, and therefore in their implications on plant-herbivore interactions and on decomposition.

3.2. Introduction

Uncertainties on the effects of elevated [CO_2] on plant ecophysiology in general, and on plant chemical composition in particular, rise because predictions have mainly been extrapolated from studies on short-term exposure of plants in manipulated environments (greenhouses and growth chambers), without considering factors such as long-term acclimation, species and genotype responses, and the complex environmental field conditions (including heterogeneous resource availabilities). Moreover, vegetation is not likely to be suddenly subjected to a dramatic increase in atmospheric [CO_2] such the one typically imposed in short-term CO_2-enrichment experiments. Different physiological processes could adjust or acclimate to gradual increases in CO_2 at different rates over periods ranging from hours to years, to generations (Eamus and Jarvis, 1989; Saxe *et al.*, 1998). Despite the potential long-term acclimation, however, few studies on CO_2 have extended beyond a single growing season. Interestingly, in some of the few relatively long studies the initial reductions in the leaf concentrations of nitrogen (N) and other elements have been found to gradually disappear over the forthcoming years (Peñuelas and Matamala, 1990; Peñuelas *et al.*, 1997). Natural CO_2 springs offer an opportunity to overcome these sources of uncertainty. They allow to study long-term responses of entire plant communities to enriched [CO_2] in complex field conditions (Miglietta *et al.*, 1993; Körner and Miglietta, 1994; Hättenshwiler *et al.*, 1997; Peñuelas *et al.,* 2001).

The steady increase in atmospheric CO_2 concentrations is likely to affect biota by producing changes not only in plant growth and allocation, but also in plant tissue chemical composition (Peñuelas and Estiarte, 1998; IPCC, 2001). Among such composition changes, most source-sink hypotheses assume that elevated CO_2 concentrations promote a relative increase of carbon availability that is accumulated in TNC and CBSSC when the provided carbon amounts exceed growth requirements (when the ratio source-sink raises) (Bryant *et al.*, 1983; Herms and Mattson, 1992; Peñuelas and Estiarte, 1998). These hypotheses predict thus a larger accumulation of carbon-based secondary and structural compounds (CBSSC), like phenolics, terpenes, or structural polysaccharides (cellulose, hemicellulose and pectin), at elevated CO_2 concentrations (Peñuelas and Estiarte, 1998). Such changes could have significant consequences for ecosystem functioning e.g. through plant-herbivore interactions (Lincoln *et al.*, 1993; Lindroth, 1996) and plant litter decomposition (O'Neill and Norby, 1996).

However, experimental results on leaf chemistry only provide evidence of increases in concentrations of soluble phenolics and condensed tannins, but not in other CBSSC with different metabolic pathways (Peñuelas and Estiarte, 1998) and overall effects on litter quality appear to be

smaller than it was initially thought (Ball, 1997). The changes in soluble phenolics and condensed tannins seemed more evident when the concentrations were expressed on structural dry weight basis, because the expression of concentration on a dry weight basis can mask or diminish the changes in the concentration of compounds. This happens especially in high-CO_2 grown plants that typically have large increases in total nonstructural carbohydrates (TNC) (Poorter *et al.*, 1997; Koricheva *et al.*, 1998), and specially in organs such as leaves with large daily TNC fluctuations due to TNC accumulation during light period and TNC export at night (e.g. Hendrix and Grange, 1991).

Plant communities of Mediterranean-type ecosystems are expected to be particularly sensitive to ongoing increases in concentrations of atmospheric CO_2 (IPCC, 2001). These Mediterranean plant communities are likely to face more severe drought conditions in the future, due to the increase in mean temperature and potential evapotranspiration and to the concurrent decrease in precipitation at Mediterranean latitudes as forecasted by General Circulation Models (Kattenburg *et al.*, 1996).

In this study, we compared leaf concentrations of phenolics and other CBSSC such as lignin, cellulose, TNC, and lipids in M*yrtus communis* L., *Erica arborea* L., and *Juniperus communis* L., Mediterranean shrubs growing in the proximity of a natural CO_2 spring, with those of comparable shrubs of the same species grown nearby but exposed to ambient [CO_2]. The objective of our research was to study changes in leaf concentrations of phenolics and other CBSSC in response to CO_2 enrichment during long-term growth in natural field conditions. Our questions were 1) whether increased CBSSC concentrations occur at elevated [CO_2] or not (i.e, whether CBSSC concentrations follow the source-sink hypotheses or, alternatively, there are long-term adjustments or "acclimation" of leaf CBSSC concentrations over a period of several generations growing on complex environmental natural conditions; and 2) if CBSSC concentrations do increase, whether they are general or particular for different compound classes and different plant species, and whether they are linked to changes in concentrations of total non structural carbohydrates or lipids.

3.3. Materials and Methods

Site description and plant material

Leaf samples were obtained from *Erica arborea, Myrtus communis* and *Juniperus communis*, three common and widespread Mediterranean macchia shrub species growing near a natural CO_2 spring called "I Borboi" in Lajatico (Pisa, Italy) (43°26'N, 10°42'E) that has been active for centuries (city council archives). A full description of the geology of the site can be found in Panichi and Tongiorgi (1975). The CO_2 enriched area extends over an area of 0.7 ha. The studied coppiced stand is dominated by *Quercus ilex* L. *Quercus pubescens* Willd. and *Arbutus unedo* L. Several other tree species (e.g. *Quercus cerris* L. and *Fraxinus ornus* L.) are represented by scattered individuals. Shrubs include the species studied here, as well as *Smilax aspera* L., *Cytisus scoparius* L., *Cistus salvifolius* L., *Genista* sp., *Ligustrum vulgare* L., *Pistacia lentiscus* L. and *Phillyrea latifolia* L. The CO_2 spring is located on the north-facing slope (20 %) of a hill near the bottom of a small valley about 200 m above sea level (Raiesi, 1998a,b; Tognetti, 1999). Almost pure CO_2 emissions occur from a series of vents located along a narrow seasonal creek and the [CO_2] tends to decrease upslope; see Tognetti *et al.* (2000a) for a map of the site. The vents emit small amount of H_2S, but their concentrations never exceed a level of 0.04 μmol mol^{-1}, which is not considered harmful to plants (Raiesi, 1998a,b; Schulte, 1998). Plants around the CO_2 spring are exposed to daytime [CO_2] of about 700 μmol mol^{-1} throughout the year, with short-term variations between 500 and 1000 μmol mol^{-1} depending on wind speed and convective turbulence. The [CO_2] varies little between different heights within the canopy (Hättenshwiler *et al.*, 1997).

Leaf samples were collected from individuals growing close to the CO_2 spring. Control measurements were made at a site chosen along the same creek, about 150 m upstream; thus root systems of the sampled plants experienced similar soil environment. The area has non-calcareous, brown loamy clay soils, developed from calcareous marl (pH 6-7), with total soil N and C/N ratio in the forest floor and mineral horizon (0-10 cm) being comparable in both the spring and control sites (Raiesi, 1998a,b). The climate is typical Mediterranean, with cool, wet winters and hot, dry summers (Tognetti, 1999). At both sites, six replicate shrubs of similar exposure were selected for each species and sampling month. Measurements were made on current year, well-developed leaves from sunny shoots in the upper third of the canopy. Mean leaf longevity of the three species is 2-3 years, buds break in April-May and leaf abscission takes place also in April-May.

To avoid possible CBSSC differences due to phenological effects, we sampled plants and leaves in the same stage of development in the two sites.

Leaf chemical composition

Every month from October 1996 through September 1997, shoots were collected early in the morning from six individuals (not necessarily the same ones every month) of each species (*Erica arborea*, *Myrtus communis* and *Juniperus communis*) at each site (CO_2 spring and control), early in the morning. Shoot material was oven-dried at 60 °C to constant weight (dry weight, DM). Thereafter, 10-12 leaves of the same stage of development were bulked for each plant at each sampling date and then ground to a fine powder in a mill. Phenolics were analyzed every month and the rest of CBSSC, TNC and lipids every two months.

Leaf concentrations of phenolics were measured on sub-samples (about 50 mg) of ground leaves. Total phenolic compounds were analyzed by Folin-Ciocalteu method, improved by using a blank of polyvinylpolypyrrolidone (PVPP) (Marigo, 1973). PVPP removes phenolic compounds from the solution and avoids overstimation of total phenolics due to non-phenolic Folin-Ciocalteu reactive compounds. Gallic acid was used as a standard to estimate concentrations of phenolic compounds.

Leaf concentrations of lignin, cellulose, hemicellulose, TNC, and total lipids were determined on subsamples of ground leaves using near infrared reflectance spectroscopy (see Joffre *et al.*, 1992 and Damesin *et al.*, 1997 for a description of the procedure). All samples were scanned with a NIRSystem 6500 spectrophotometer. The spectral and wet chemical database used to build calibration equations comprises leaves of 25 species, representing the diversity of Mediterranean woody species collected by us from Mediterranean areas of Portugal, Spain, France and Italy and includes part of the database of Meuret *et al.* (1993). The concentrations of fiber, total lipids and TNC in these calibration set samples were determined using wet chemistry methods. Fiber fractions were determined using the Fibertec procedure (Van Soest and Robertson, 1985), total lipids were obtained by weighing the residue extracted by a chloroform-methanol mixture (see Allen, 1989) and TNC analysis was carried out following the method of Farrar (1993).

Statistical analyses

Within each species, two-way analyses of variance (ANOVA) with sampling date and site (with different [CO_2]) as the main effects were conducted for all compound concentrations after testing for normality and homogeneity of variance. A repeated-measures analysis was not considered appropriate because we did not sample the same shrubs over the sampling time period. Differences amongst the three species were also tested with ANOVA. Statistical analyses were conducted using Statview 4.5 (Abacus Concepts Inc., Berkeley, CA, USA) and SYSTAT 5.2 (SYSTAT Inc., Evanston, IL, USA).

3.4. Results and Discussion

There were significant differences amongst species for most CBSSC ($P < 0.01$, ANOVA) (Fig. 12-13). *Myrtus communis* had the greatest leaf concentrations of phenolics and the lowest leaf concentrations of hemicellulose (Fig. 12-13). *Erica arborea* had the greatest leaf concentrations of hemicellulose and the lowest leaf concentrations of TNC. *Juniperus communis* had the greatest leaf concentrations of lignin, cellulose and lipids and the lowest leaf concentrations of phenolics (Fig. 12-13).

There were different responses to site (and therefore [CO_2]) depending on compound and species. Foliar phenolic concentrations were 28% lower in the CO_2 spring site than in the control site in *Erica arborea*. Foliar TNC concentrations were 13% greater in *Myrtus communis,* but on the contrary, 12% lower in *Juniperus communis*. Foliar lignin concentrations were 5% greater in *Juniperus communis*. Leaf concentrations of hemicellulose were 19% greater in *Myrtus communis*. For all other compounds and species there were no significant patterns in response to the elevated [CO_2] of the spring site (Fig. 12-13).

Concentrations of many CBSSC differed significantly by sampling month (Fig. 14 for phenolics) as expected for leaves in different developmental stages (Peñuelas and Estiarte, 1998). However, the three shrub species did not display consistent seasonal changes for most CBSSC, except for a slight trend towards minimum values in spring (youngest leaves) and maximum values in autumn-winter (mature leaves) (Fig. 14). This trend was the strongest for *Myrtus communis*. The analyses revealed some statistically significant interactions between CO_2 enrichment and month (Fig. 14).

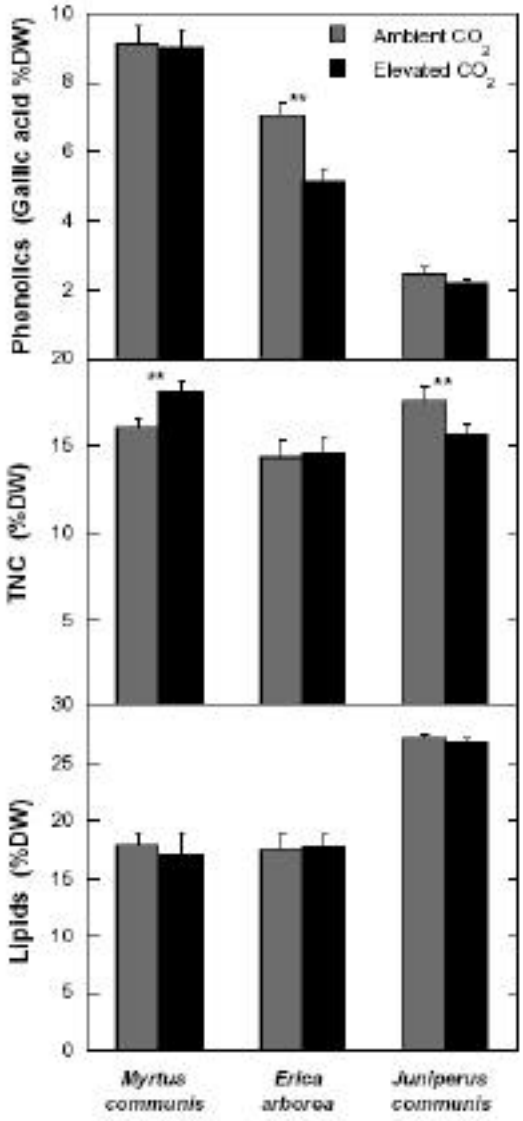

Figure 12. Effects of elevated [CO_2] on annual average leaf phenolics (top), total non-structural carbohydrates (TNC) (middle) and lipid concentrations (bottom). Data presented as percent dry weight (%DM). Error bars indicate ±SEM. n=6 month averages, calculated on 3 plants (10-12 leaves pooled together for each plant). ** $p<0.01$, ANOVA.

Absence of increased CBSSC concentrations at elevated [CO_2]

The most important finding of this study is that although long-term growth at high concentrations of atmospheric CO_2 generated some differences in the CBSSC composition of plant leaves depending on species and compounds, the overall CBSSC concentrations did not increase in the CO_2 spring site. Even lower phenolic concentration in plants grown at the CO_2 spring site than at the control site were found for one of the studied species, *Erica arborea*. Moreover, these results

were not due to a dilution effect since there was not either increased structural or non-structural carbon or lipid concentrations (Fig. 12).

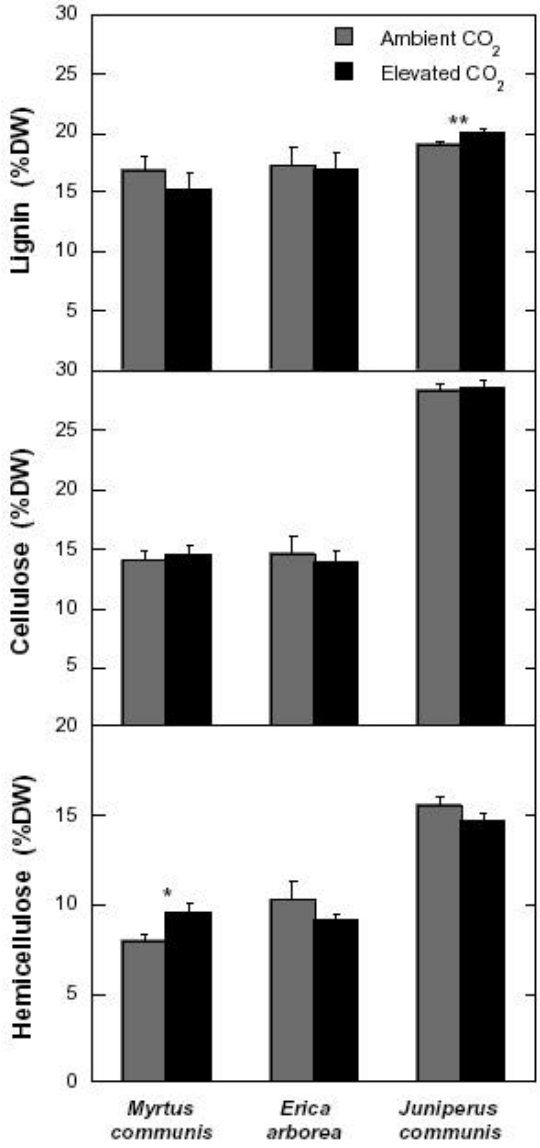

Figure 13. Effects of elevated [CO_2] on annual average leaf lignin (top), cellulose (middle) and hemicellulose (bottom). Data presented as percent dry weight (%DM). Error bars indicate ±SEM. n=6 month averages, calculated on 3 plants (10-12 leaves pooled together for each plant). *p<0.05, ** p<0.01, ANOVA.

These total phenolic data contrast to several previous CO_2 experimental studies that were conducted in less natural, shorter-term conditions (Peñuelas and Estiarte, 1998). However, the results for lignin and structural polysaccharides, both carbon based structural compounds that are linked to cell wall, are in agreement with available data (Peñuelas and Estiarte, 1998). All the data are in apparent disagreement with carbon source-sink hypotheses that predict increased CBSSC concentrations at elevated CO_2 concentrations (Peñuelas and Estiarte,

1998). These hypotheses assume that elevated CO_2 concentrations promote a relative increase of carbon availability, that is accumulated in TNC and CBSSC when the provided carbon amounts exceed growth requirements (when the ratio source-sink raises) (Bryant *et al.*, 1983; Herms and Mattson, 1992; Peñuelas and Estiarte, 1998).

Interspecific and environmental heterogeneity

It is difficult to ascertain the specific cause or causes of the absence of elevated CO_2 effect on CBSSC concentrations from the available data. Several explanations can be proposed. A likely explanation for the lack of CO_2 sensitivity may be related to the various carbon investment strategies available to different plant species, including growth, storage, structural compound and defense components. The interspecific variability of our data is not surprising; it is also found in many other ecophysiological variables including growth, whose response to elevated CO_2 can vary greatly even in co-occurring species of the same functional type and in interaction with abiotic and biotic factors (Bazzaz, 1990; Körner, 2000). Diverse interspecific responses have also been found for concentrations of several elements in these same plants. Under the elevated [CO_2] of the spring site, both greater (e.g. in Ca, K, S, Mg, Mn, Al, Fe, P and Ti) and lower (e.g. in C, Ba, Co, N, Cr, Sr, P and B) leaf concentrations have been reported. Apart from interspecific differences, there were also different CO_2 responses among the different elements and the seasons of the year (Peñuelas *et al.*, 2001). These interspecific and seasonal differences show that different plant species may use different available resources within Mediterranean sites and seasons (Tognetti and Peñuelas, 2001). In fact, the complex natural conditions of the field make it difficult to find exactly identical sites for resource availability or environmental conditions, and under such natural conditions, variability in characteristics within and between plant populations can exceed any response to CO_2 (van Gardingen *et al.*, 1997).

Long-term acclimation

Another explanation for these results comes from a likely long-term adjustment or acclimation of photosynthetic carbon uptake, and finally of most leaf CBSSC concentrations to elevated CO_2 over many generations of plant development. Plants have grown in such CO_2 enriched environment at least for several centuries (city council archives). Provided that stomatal conductance of plants grown at the CO_2 spring site was lower than in control plants (Tognetti *et al.*, 2000), the studies of the ^{13}C composition of these plants suggest photosynthetic acclimation

under long-term CO_2-enriched atmosphere (Miglietta *et al.*, 1998; Tognetti and Peñuelas, 2001) leading to decreased photosynthetic capacity (Miglietta *et al.*, 1998). We have no photosynthetic data for these shrubs, but at the same site, the evergreen *Quercus ilex* showed significant down regulation and homeostatic adjustment to elevated CO_2 (W.C. Oechel and C.L. Hinkson, personal communication) in accord with the results of Migglietta *et al.* (1995) and Oechel and Vourlitis (1996). The down-regulated photosynthetic rates may have been enhanced by water stress, which may have been sufficient to decrease carbon uptake relative to the other elements (i.e. restrict growth more than nutrient uptake). The Mediterranean sclerophylls studied in this experiment may have an intrinsic growth strategy that highly prioritizes water saving over carbon uptake. In fact, other forest tree species of these sites did not exhibit any increase in aboveground productivity. Possible acclimations to the high CO_2 or/and nutrient limitations have been suggested to counteract the positive effect of CO_2 under drought stress (Tognetti *et al.*, 2000a).

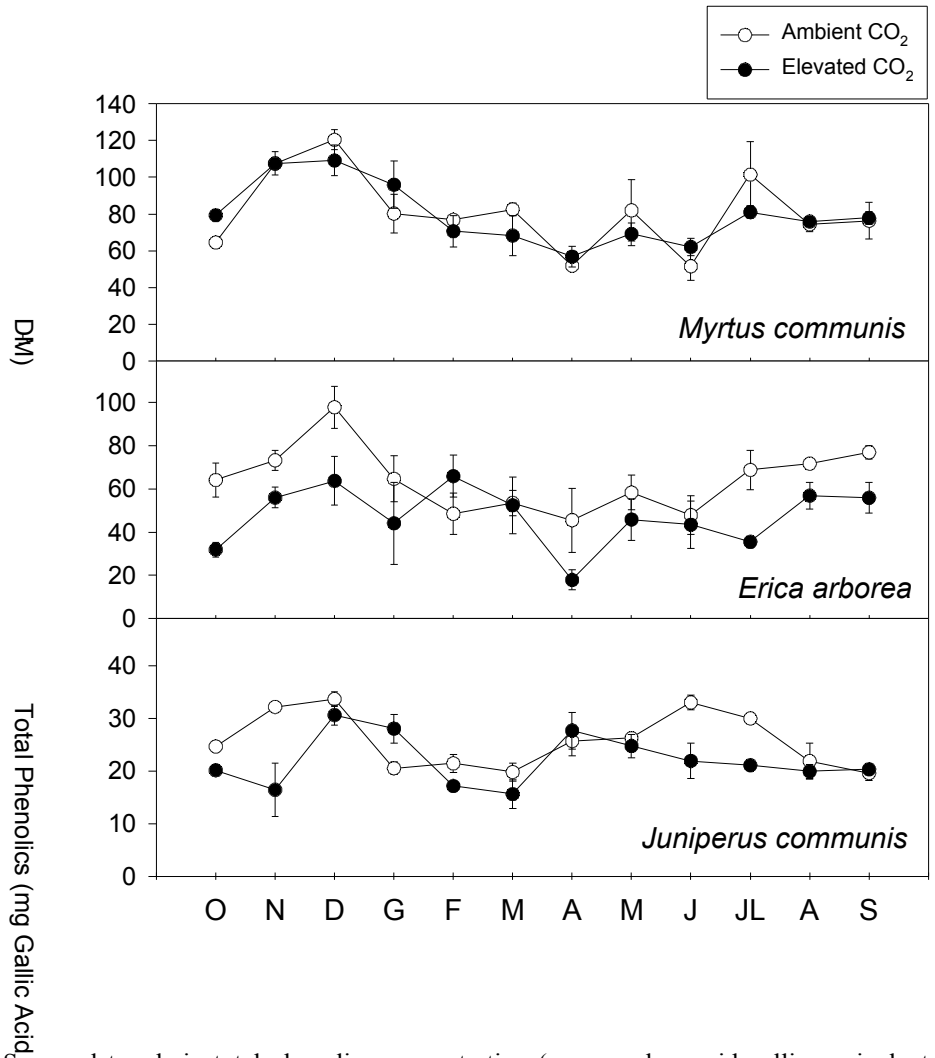

Figure 14. Seasonal trends in total phenolics concentration (expressed as acid gallic equivalents) measured in leaves of *M. communis*, *E. arborea* and *J. communis* plants during 1996-1997. Vertical bars indicate standard error of the mean. $n = 3$ plants (10-12 leaves pooled together for each plant).

Complex space and time conditions

These results showing minimal or species-specific time-dependent changes are especially valuable because they were obtained accounting for the complexity of interactions in real life throughout time and space (plant age, neighbors, microbial partners, soil processes, atmospheric conditions…) (Körner, 2000). These results showed that increased CBSSC is not a general response to long-term exposure to elevated [CO_2] in these shrubs. This conclusion is in apparent disagreement with the carbon source-sink hypotheses. Some of the premises of such hypotheses may be unaccomplished under natural field conditions (e.g. microhabitats are heterogeneous for resource availabilities). Moreover, these hypotheses partially ignore the homeostatic nature of organisms and their evolutive history (Hamilton *et al.*, 2001). Our data is in support of acclimation to elevated [CO_2] of carbon metabolism, with relative lack of carbon excess. This acclimation in leaf chemical composition under elevated CO_2 concentration of spring site emphasizes, once more, the importance of conducting natural (field conditions) long-term experiments with plants under elevated [CO_2]. Long-term studies allow the record of homeostatic plant mechanisms and possibly adaptive evolutionary histories. Different growth rates (carbon sink) under different interacting environmental conditions or resource availabilities and under seasonal and specific variation must be considered, apart from [CO_2] itself, to study the carbon-nutrient-water relationships under elevated [CO_2] in shrubs of Mediterranean environments.

Caution on possible implications for herbivory and decomposition

Many authors have noted the potential significance of CO_2-induced changes in leaf nutrient quality for herbivore feeding and development (Fajer *et al.*, 1989; Lincoln *et al.*, 1993; Lindroth *et al.*, 1993; Arnone *et al.*, 1995; Peñuelas and Estiarte, 1998) and for litter decomposition and carbon sequestration in soils (Strain, 1985; Rastetter *et al.*, 1992; Comins and McMurtrie, 1993; Schimel, 1995; Peñuelas and Estiarte, 1998). Plant digestibility and litter decomposition would be hindered by increased CBSSC concentrations under elevated [CO_2]. However, the results of this investigation suggest caution in attributing increased CBSSC concentrations to elevated [CO_2], and in turn, consequences for herbivory and decomposition under long-term CO_2-exposure, which is especially relevant on the context of the global carbon budget.

Acknowledgements

We thank financial support from CICYT grants CLI99-0479 and REN-2000-0278/CLI (Spanish Government), IMMPACTE grant (DURSI and DMA, Catalan Government) and Fundació Territori i Paisatge (Catalonia).

RESULTATS (II)

EFECTES DELS FENOLS EN EL CICLE DEL N

Capítol 4

4. Interaction between the phenolic compound bearing species *Ledum palustre* and soil N cycling in a hardwood forest

E. CASTELLS [1], J. PEÑUELAS [1], D. W. VALENTINE [2]

[1] Unitat d'Ecofisiologia CSIC-CREAF, Centre de Recerca Ecològica i Aplicacions Forestals (CREAF), Facultat de Ciències, Universitat Autònoma, Bellaterra 08193 (Barcelona), Spain
[2] Department of Forest Science, University of Alaska Fairbanks, Fairbanks AK 99775-7200, USA

(Sotmès a publicació)

4.1. Abstract

The effects of the understory shrub *Ledum palustre* on soil N cycling were studied in a hardwood forest of Interior Alaska. This species releases high concentrations of phenolic compounds from green leaves and decomposing litter by rainfall. Organic and mineral soils sampled underneath *L. palustre* and at nearby control sites were amended with *L. palustre* litter leachates and incubated at controlled conditions. We aimed to know (i) whether *L. palustre* presence and litter leachate addition changed net N cycling rates in organic and mineral soils, and (ii) what N cycling processes, including gross N mineralization, N immobilization and gross N nitrification, were affected in association with *L. palustre*.

Our results indicate that N transformation rates in organic soils were not affected by *L. palustre* presence or leachate addition. However, mineral soils underneath *L. palustre* as well as soils amended with leachates had significantly higher C/N ratio and microbial respiration, and lower net N mineralization and N-to-C mineralization compared to control soils. No nitrification was detected. Plant presence and leachate addition also tended to increase gross N mineralization and ammonium consumption. These results suggest that soluble C compounds present in *L. palustre* increased N immobilization in mineral soils when soil biota used them as a C source. Increases on gross N mineralization could be caused by an enhanced microbial biomass due to C addition. Since both plant presence and leachate addition decreased soil C/N ratio and had similar effects on N transformation rates, our results suggest that litter leachates could be partially responsible for plant presence effects. The lower N availability under *L. palustre* canopy could exert negative interactions on the establishment and growth of other plant species.

4.2. Introduction

Presence of plants has been shown to influence small-scale patterns of nutrient availability in various ecosystems by changing the quantity and quality of organic matter in the nearby soil (Chen and Stark, 2000; Smith *et al.*, 1994). These effects are species-specific since plants differ in litter production (Hobbie, 1992) and litter tissue chemical composition (Binkley and Valentine, 1991). Thus, soil underneath plants can have more organic matter and soil microbial activity compared to bare ground (Aguilera *et al.*, 1999; Hook *et al.*, 1991; Vinton and Burke, 1995) although in some cases plant presence had no effect on soil organic matter (Jackson and Caldwell, 1993) or changes in organic matter were not related to changes in microbial activity (Chen and Stark, 2000; Jackson and Caldwell, 1993). Litter chemical traits such as C:N ratio, lignin:N ratio and phenolic compound concentrations have been inversely related to decomposition, and thus to the release of inorganic nutrients by microbes (Hättenschwiler and Vitousek, 2000; Hobbie, 1992).

Phenolic compounds may play a dominant role in controlling many aspects of plant-soil interactions, specially those related with organic matter dynamics and nutrient cycling (Kuiters, 1990; Northup *et al.*, 1998; Schimel *et al.*, 1995). One of the most characteristic properties of phenolic compounds, including the high molecular weight condensed tannins, is their capacity to form recalcitrant complexes with proteins and thus to alter the pool and form of nutrients (Hättenschwiler and Vitousek, 2000). When these complexes occur, mainly during senescence, leaf N is initially immobilized and gradually released in the course of decomposition (Kuiters, 1990). For instance, phenolic concentrations have been correlated with slower soil organic matter decomposition and turnover rates (Horner *et al.*, 1988; Nicolai, 1988; Palm and Sanchez, 1990), higher N immobilization rates in litter (Gallardo and Merino, 1992) and lower N mineralization rates in litter and organic soil (Fox *et al.*, 1990; Northup *et al.*, 1995; Palm and Sanchez, 1990; Palm and Sanchez, 1991; Schimel *et al.*, 1995). Moreover, phenolic compounds have been also shown to change microbial activity by stimulating microbial population growth, microbial respiration and N immobilization (Blum, 1998; Blum and Shafer, 1988; Boulfalis and Pellissier, 1994; Shafer and Blum, 1991; Sparling *et al.*, 1981; Sugai and Schimel, 1993), and inhibiting microbial respiration (Boulfalis and Pellissier, 1994; Schimel *et al.*, 1995) or nitrification (Rice, 1984). The presence and importance of each one of these effects seems to highly depend on the molecular size, structure and concentrations of phenolic compounds (Boufalis and Pellisser, 1994; Schimel *et al.*, 1995). Thus, condensed tannins seem more involved in slowing degradation processes when they form stable

complexes with proteins and inorganic N (Northup *et al.*, 1995; Palm and Sanchez, 1990), whereas phenolic acids are easily degraded by microbes (Blum, 1998; Blum and Shafer, 1988; Sugai and Schimel, 1993). Because the overall effects of phenolic compounds on N cycling are a decrease in net N mineralization, either by decreasing gross mineralization or nitrification, or by increasing immobilization, the use of ^{15}N pool dilution techniques for estimating gross N transformation rates is adequate to elucidate what processes are being affected (Hart *et al.*, 1994).

The presence and concentration of plant phenolic compounds, and thus the potential effects of their release to the nearby soil, is highly dependent on the plant species and the abiotic environment (Bryant *et al.*, 1983; Hamilton *et al.*, 2001; Peñuelas and Estiarte, 1998). The ecological relevance of phenolic compounds can be of especial interest in N-limited ecosystems such as boreal ecosystems, where slow growing species with high concentrations of carbon-based secondary compounds predominate. However, it is still not clear whether changes in N cycling related to plant phenolic compounds can be found in natural conditions since these effects have been mainly tested in laboratory experiments with individual compounds. High amounts of phenolic compounds can be released by rainfall from green foliage and decomposing litter (Gallet and Pellissier, 1997; Harborne, 1997; Inderjit and Mallik, 1996a; Kuiters and Sanrink, 1986) and thus affect nutrient cycling.

Ledum palustre is a late successional evergreen shrub widely distributed in boreal ecosystems that readily leaches high concentrations of soluble phenolic compounds into water. We selected this species in order to study whether leaf leachates could be responsible for the effects of plant canopy on N cycling in natural conditions. Organic matter content, soil respiration and net N mineralization were measured in organic and mineral soils and gross N mineralization was also measured in mineral soils sampled underneath *L. palustre* in a hardwood forest. Soils were amended with *L. palustre* litter leachates and incubated in the laboratory. Because mineral soil is also influenced by the above organic horizon we also characterized the organic horizon carbon quality (lignin, cellulose and condensed tannins) to look for differences in the soil decomposability properties. The objectives of the present study were i) to determine whether *L. palustre* presence and *L. palustre* leachates addition changed soil N dynamics, and ii) to determine the N cycling specific processes that were affected, including changes in mineralization, nitrification or immobilization. We wanted to know whether *L. palustre* leachates could be responsible for the potential plant presence changes on N cycling. Besides affecting site chemical quality, plant secondary compounds of some ericaceae species, including *Ledum groenlandicum*, have been shown to difficult the

establishment and growth of other plants species (Inderjit and Mallik, 1996a,b). In this study, we finally discuss whether the chemical interactions between *L. palustre* and other plants could be related to decreases in soil N availability for plant uptake or otherwise to direct effects through allelopathic processes.

4.3. Materials and methods

Site description

The study sites were located in a south-facing mixed hardwood forest in Caribou Poker Creek Research Watershed (North of Fairbanks, Interior Alaska, USA). Soils are silt loam, well drained with no permafrost, and belonging to Olnes series. Dominant species are aspen (*Populus tremuloides*), alaskan paper birch (*Betula resinifera*) and black spruce (*Picea mariana*). *Ledum palustre* is a late successional evergreen shrub which is distributed forming patches of moderate density which seem to be located in more open areas into the forest. During summer 1999 we randomly selected five *L. palustre* patches and five adjacent control plots. A $1m^2$ quadrat was located in the middle of the *L. palustre* patch in order to avoid edge effects, and in the selected non-Ledum sites. Because black spruce can also leach phenolic compounds by rainfall we avoided this species close to the selected plots. Biomass of *L. palustre* was estimated by sampling shoot new growth within the 1 m^2 quadrat. Average density of *L. palustre* was 35.58 ± 6.64 g m^{-2} and it ranged from 18.75 to 59.91 g m^{-2} in the different plots although no significant differences were detected among sites. Biomass of the other understory species in both Ledum and non-Ledum sites was determined by sampling all rooted plants in three 20 cm^2 quadrats per each plot. Feathermoss, *Polytricum sp* and *Vaccinium vitis-ideae* were the dominant understory species, followed by *Lycopodium annotinum*, *Lycopodium complanatum*, *Cornus sp*, *Epilobium sp* and *Equisetum silvaticum*. Total understory biomass other than *Ledum palustre* was 49.57 ± 6.635 g m^{-2} for Ledum sites and 28.49 ± 14.95 g m^{-2} for control sites. There were no statistical differences between sites for total biomass (Table 7) as well as for biomass of each individual species (data not shown).

Preparation of L. palustre leachates

In order to study the global effects on nutrient dynamics of the high variety of phenolics and other carbon-based compounds present in plant species we used foliage leachates instead of purified phenolic compounds. Litter leachate was selected instead of green leaves leachate because release of soluble phenolic compounds have been documented to be larger than from leaves (Kuiters, 1990). Standing leaf litter of *L. palustre* corresponding to previous year production was sampled at the study sites described above. Leachate was obtained by shaking fresh litter (25 g of equivalent DM) in 1 L distilled water at room temperature for 24h (Zackrisson and Nilson, 1992) and filtered through filter paper Whatman 42. Distilled water was used as a control. Leachates were analyzed for total phenolics by the Folin-Ciocalteu method (Marigo, 1973) using gallic acid as a standard (767 mg L^{-1}), and for dissolved organic carbon (DOC) (1222 mg L^{-1}) (Shimadzu TOC 5000).

Soil sampling and incubations

Organic horizon (Oe and Oa) and the top 7 cm of mineral soil were sampled in three different localizations inside the $1m^2$ quadrat, and every horizon was bulked together within a site. Organic and mineral horizons were sieved with 5.6 and 2 mm mesh respectively and kept at 4 C before incubating. Bulk density of organic (0.11 g cm^3) and mineral soils (0.55 g cm^3), and pH of mineral soil (4.9) were not significantly different between Ledum and non-Ledum sites.

Soils sampled under *L. palustre* and in nearby control sites were incubated in at 15 ºC during 30 days. Organic (20 g FW) and mineral soil (50 g FW) were placed into 250 mL jars provided with septa which allowed to take air samples, and they were amended with litter leachate or distilled water (4 and 6 mL for organic and mineral soils respectively) just before starting the incubation. Additional distilled water was added to the soils until field capacity, which was measured from a subsample using a modified procedure from Tan (1996). Soil respiration was measured every week by analyzing CO_2 accumulated in the jars by a Gas Chromatograph (Shimadzu GC-14A), and thereafter jars were opened and ventilated and distilled water was added to maintain field capacity when necessary. Net N mineralization rates were estimated by analyzing initial and final ammonium and nitrate concentrations. Soils (15 g FW) were extracted with 75 mL of 2 M KCl for 1 h and analyses of ammonium and nitrate were conducted on a modified Technicon AutoAnalyzed II system. Net N mineralization rates were calculated subtracting initial concentrations from final

concentrations and expressed per unit of soil dry weight or organic C. Total C and N were analyzed at the end of incubation using CNS analyzer (LECO Model 200). A subsample of the organic horizon was dried at 65 ºC, ground and analyzed at the University of Alaska Palmer Research Station for acid-detergent fiber (ADF), neutral detergent fiber (NDF), cellulose, hemicellulose and lignin using the Van Soest procedure (Goering and Van Soest, 1970). Condensed tannin concentrations were analyzed by the proantocyanidin method (Waterman and Mole, 1994).

Gross N transformation rates

Gross rates of N mineralization and ammonium consumption in mineral soils were determined by $^{15}NH_4^+$ isotope dilution (Hart *et al.*, 1994). A solution containing 6 mL of leachate or distilled water and 3 mL of $^{15}NH_4^+$ (containing 0.05 mg ^{15}N 99 atom %) were added to 50 g of soil. Additional distilled water was added until field capacity when necessary. Samples were homogenized during 5 min and thereafter 5 g of soil was immediately extracted with 2 M KCl in order to have initial inorganic N concentrations and ^{15}N recovery. The rest of the sample was incubated at 15 ºC and extracted after 24 h. Initial and final times of incubation were recorded. Inorganic N was transferred from KCl extracts to a cut filter paper Whatman 3 (Holmes *et al.*, 1998) which was analyzed for ^{15}N atom % in a isotope ratio mass spectrometer (20-20 PDZ Europa, Cheshire, UK). Gross N transformation rates were calculated from changes in inorganic N concentrations and changes in ^{15}N atom % during the incubation following Kirkham and Bartholomew (1954). Gross N mineralization rates (m) and NH_4^+ consumption (C_A) were calculated by the equations:

$$m = \frac{\left[NH_4^+\right]_0 - \left[NH_4^+\right]_t}{t} * \frac{\log\left(APE_0 / APE_t\right)}{\log\left(\left[NH_4^+\right]_0 / \left[NH_4^+\right]_t\right)}$$

$$C_A = m - \frac{\left[NH_4^+\right]_t - \left[NH_4^+\right]_0}{t}$$

where, m = gross N mineralization rate (μg N g^{-1}DM day^{-1})

C_A = NH_4^+ consumption rate (μg N g^{-1}DM day^{-1})

t = time (days)

$[NH_4^+]_0$ = total NH_4^+ concentration (μg g^{-1}DM) at time-0

$[NH_4^+]_t$ = total NH_4^+ concentration (µg g^{-1}DM) at time-t

APE_0 = atom % ^{15}N excess of NH_4^+ pool at time-0

APEt = atom % ^{15}N excess of NH_4^+ pool at time-t

where, APE = (the atom % ^{15}N enrichment of a N pool enriched with ^{15}N) - (the atom % ^{15}N enrichment of that pool prior to ^{15}N addition, i.e. the background enrichment)

We assumed the background ^{15}N enrichments to be 0.37% atom % ^{15}N (Hart *et al.*, 1994). Since no nitrification was present in the studied soils (no nitrate was detected in the initial nor in the final analyses), we will consider that ammonium consumption are mainly determined by ammonium immobilization.

.

Statistical analyses

A two-way ANOVA, one-trial repeated measures ANOVA or two-trial repeated measures ANOVA were conducted depending on the variables analyzed (see table legends for more details). *L. palustre* biomass density was used as a covariant when indicated. Data was analyzed for normality and no transformations were necessary in any case. All analyses were performed using Statistica 99 (Statsoft Inc., Tulsa, USA).

	Control site	***Ledum* site**		
	Mean ± SE	Mean ± SE	F	p
Understory biomass (g m^{-2})	28.49 ± 14.95	49.57 ± 6.63	2.56	0.19
Depth organic horizon (cm)	5.06 ± 0.31	4.97 ± 0.31	0.06	0.8
NDF (%)	61.9 ± 1.1	66.08 ± 3.14	1.34	0.3
ADF (%)	49.86 ± 1.37	53.83 ± 2.9	0.99	0.37
Hemi-cellulose (%)	12.04 ± 0.69	12.26 ± 0.48	0.13	0.73
Cellulose (%)	12.51 ± 1.36	13.89 ± 1.58	0.24	0.64
Lignin (%)	21.82 ± 1.04	22.05 ± 1.65	0.01	0.92
Condensed tannins (mg g^{-1}DM)	nd	nd	-	-

Table 7. Means ± SE and t-test comparing understory biomass other than *L. palustre*, depth of organic horizon and organic soil chemical analyses sampled in control sites (without *L. palustre*) and Ledum sites (Under *L. palustre*). (n = 20 for organic horizon depth and n = 5 for the other variables). No significant differences were found for any variable. nd = not detectable.

4.4. Results

Organic horizons sampled underneath *L. palustre* had 3.6 % and 7.9 % lower organic C (soils unamended and amended with *L. palustre* leachates, respectively) than control soils (Fig. 15). No differences on neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, hemicellulose or cellulose were found between organic soils associated and non-associated to *L. palustre*. Condensed tannins were not present in any site (Table 7). In mineral soils, no differences were found for total organic C and N between soils underneath *L. palustre* and control sites. However, C/N ratio was significantly lower in the control sites (Fig. 15).

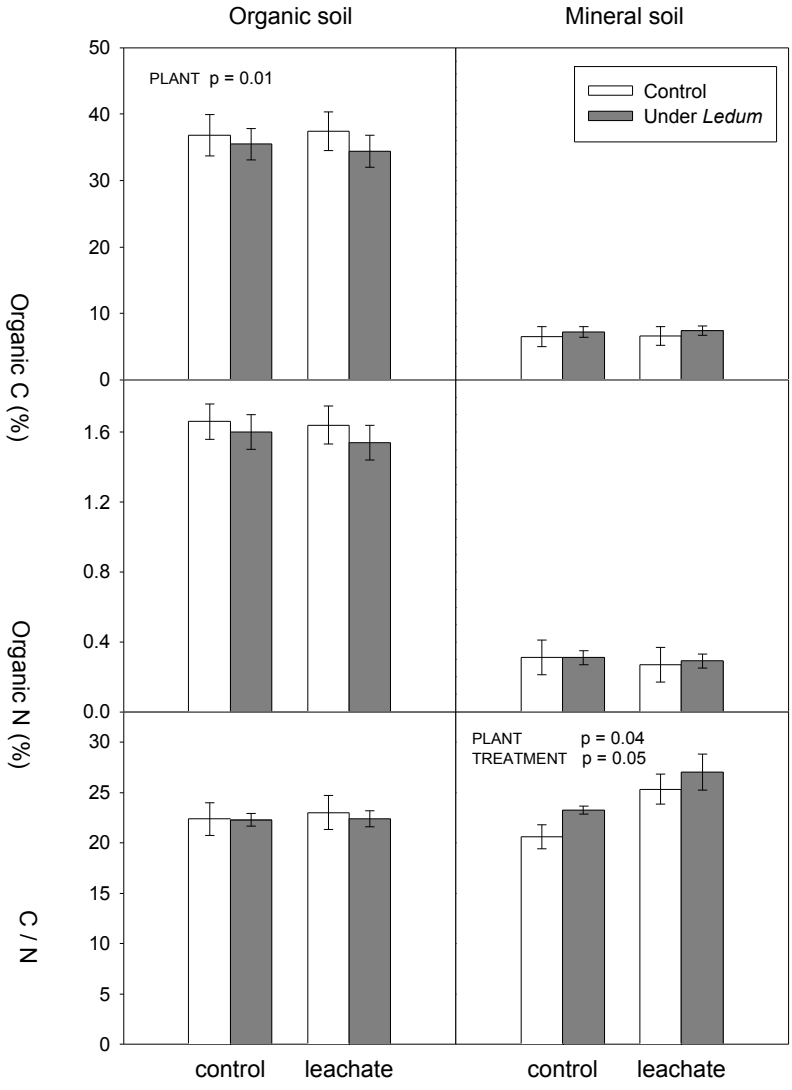

Figure 15. Effects of *L. palustre* presence and leachate addition on organic C, organic N and C/N ratio of organic and mineral soils. Values represent means and SE. We tested statistical differences for plant presence or absence ("PLANT") and amendments with leachate or distilled water ("TREATMENT") conducting a repeated measures ANOVA for PLANT (n = 5). *L. palustre* biomass density was used as a covariant. Significant p-levels are shown.

	Net N mineralization		C mineralization		N-to-C mineralization	
	F	*p*	*F*	*p*	*F*	*p*
Organic soil						
TREATMENT	0.00	0.98	0.28	0.61	0.17	0.69
PLANT	0.1	0.75	2.97	0.13	0.14	0.72
TIME	-	-	25.98	**<0.001**	-	-
TREATMENT x PLANT	0.42	0.53	0.32	0.58	0.25	0.62
TREATMENT x TIME	-	-	1.77	0.18	-	-
PLANT x TIME	-	-	1.47	0.25	-	-
TREATMENT x PLANT x TIME	-	-	0.42	0.74	-	-
Mineral soil						
TREATMENT	14.03	**0.007**	0.002	0.96	10.31	**0.01**
PLANT	6.16	**0.03**	3.46	0.09	11.07	**0.01**
TIME	-	-	10.78	**<0.001**	-	-
TREATMENT x PLANT	1.4	0.26	0.05	0.81	1.48	0.25
TREATMENT x TIME	-	-	3.86	**0.02**	-	-
PLANT x TIME	-	-	0.56	0.64	-	-
TREATMENT x PLANT x TIME	-	-	0.47	0.7	-	-

Table 8. Statistical analyses for net N mineralization and respiration of organic and mineral soils sampled under *L. palustre* and in a nearby control site (PLANT). Soils were amended with leachate or with distilled water (TREATMENT). We conducted a repeated measures ANOVA (PLANT) for net N mineralization and N-to-C mineralization, and two-trial repeated measures ANOVA (PLANT and TIME) for soil respiration. TIME variable is referring at date of sampling along a one-month incubation period. Plant density was used as covariant. (n = 5) $p<0.05$ are highlighted in bold.

Presence of *L. palustre* and leachate addition had no significant effects on microbial activity of organic soils (Fig. 16, Table 8). In mineral horizons, net N mineralization on a dry weight basis was 73.4 % and 264.3 % lower (soils unamended and amended with *L. palustre* leachate, respectively) and total CO_2 production was 27.9 % and 27.8 % higher in soils sampled under *L. palustre* compared to control sites (Fig. 16, Table 8). No nitrification was detected in organic or mineral soils. Leachate addition decreased a 109.7 % and 234.2 % net N mineralization in control and underneath *L. palustre* soils, and the effects were similar in both soils since no significant interactions were found between treatment and plant presence (Fig. 16, Table 8). Although no effect of leachate addition was found for soil respiration overall the incubation period, the significant interaction between treatment and incubation time showed that leachate addition increased 48.1 % and 71.5 % soil respiration during the first week of incubation (Fig. 17, Table 8). Both soils sampled under *L. palustre* and control soils had similar responses to leachate addition for all parameters.

L. palustre sites had 310.8 % higher gross N mineralization rates compared to control sites and 110.6 % higher marginally significant ammonium consumption rates (Fig. 18). When

leachates were added to control soils, gross N mineralization increased 208.1% and ammonium consumption 151.5 % compared to unamended soils, although only the differences in ammonium consumption were significant (Fig. 18). No differences between plant presence and leachate addition were found for gross N mineralization or ammonium consumption (Fig. 18).

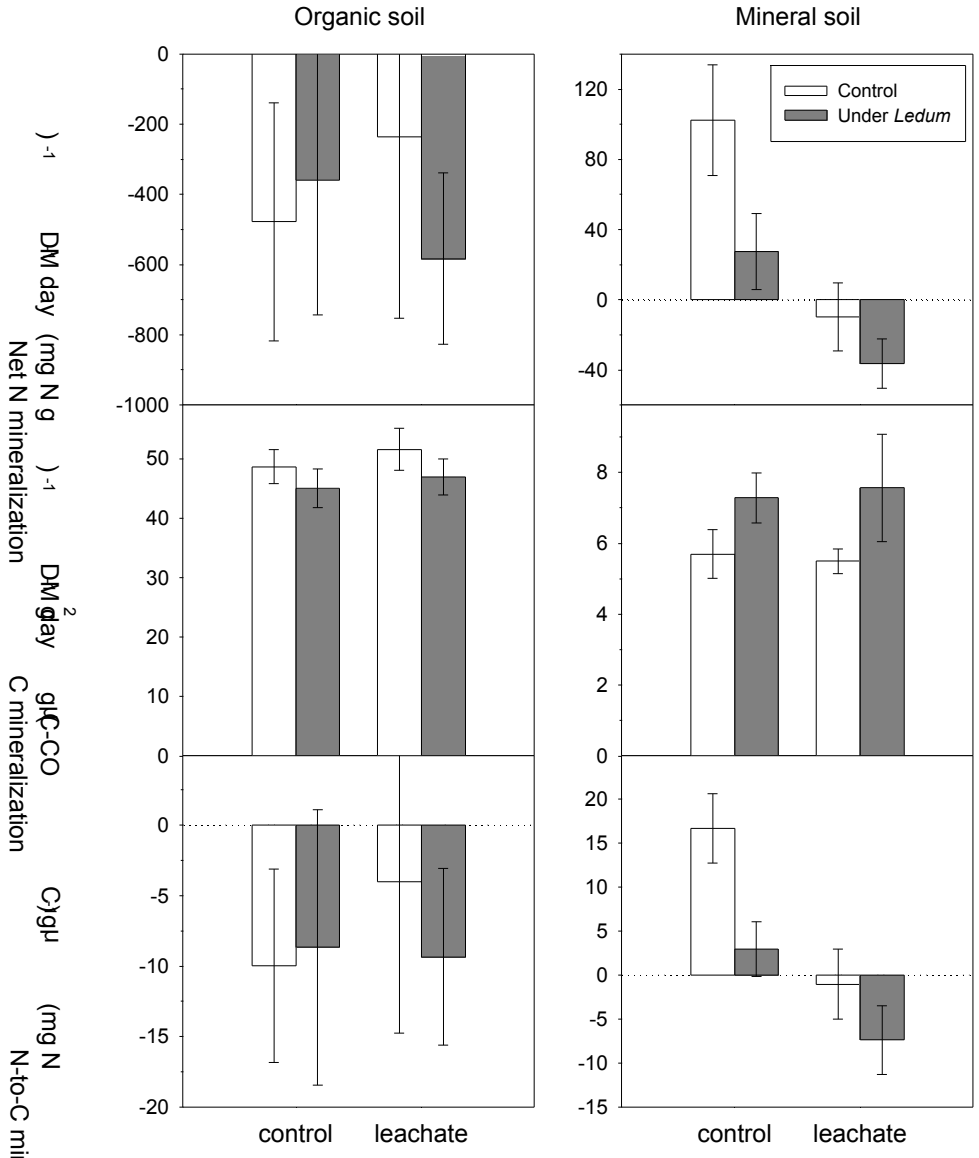

Figure 16 Net N mineralization, soil respiration and N-to-C mineralization of organic and mineral soils sampled under *L. palustre* and in nearby control sites, and amended with distilled water (control) or *L. plaustre* leachate. Values represent means and SE (n = 5).

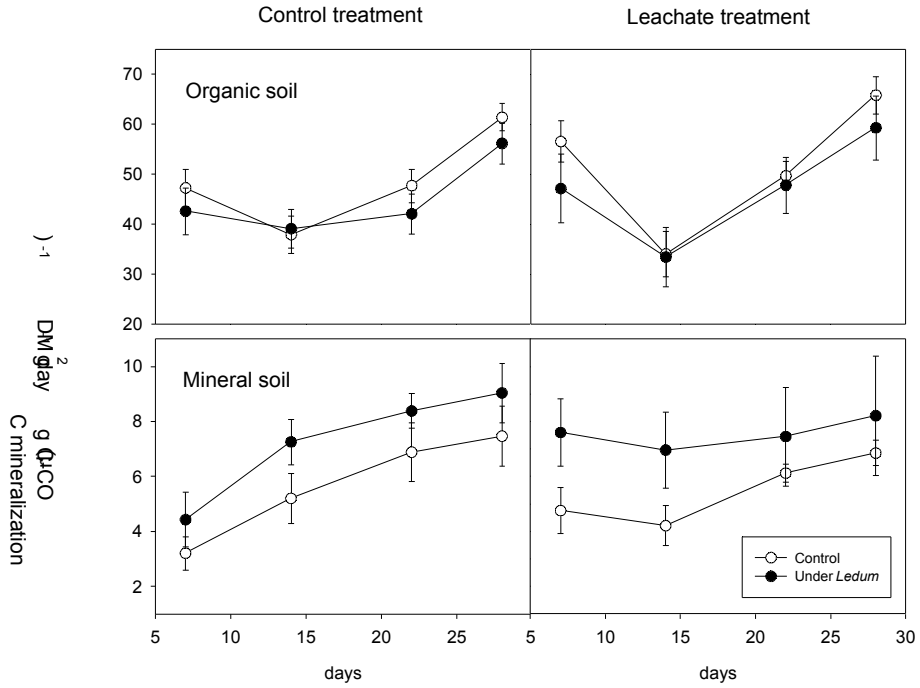

Figure 17. Changes in soil respiration along a one-month incubation for organic and mineral soils sampled under *L. palustre* and in nearby control sites, and amended with distilled water (control) or with litter leachate. Values represent means and SE (n = 5).

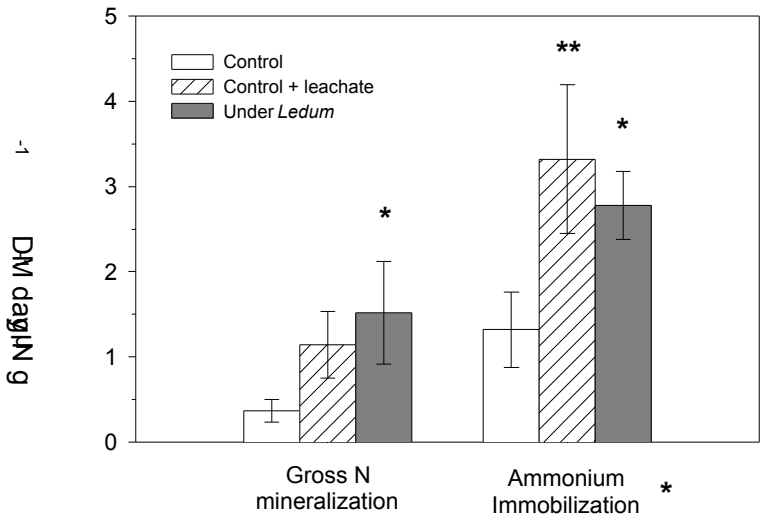

Figure 18. Gross N mineralization and ammonium immobilization rates from soils sampled under *L. palustre* and in nearby control sites, and from control site soils amended with *L. palustre* leachates. Values represent means and SE. A t-test was conducted in order to test significant differences for treatment (n = 5). *L. palustre* biomass density was used as a covariant. Post-hoc comparisons were conducted using a LSD-test. Significant levels: *p < 0.1, **p < 0.05. For post-hoc comparisons significant levels are referring to differences between control and the other treatments.

4.5. Discussion

Effects of L. palustre presence and leachate addition on N cycling

The results show that *L. palustre* canopy and litter leachate addition changed N cycling in mineral soils. Although we found no differences in organic matter content between mineral soils sampled under *L. palustre* and soils sampled in nearby control sites, both plant presence and leachate addition increased soil C/N ratio and microbial respiration, and decreased net N mineralization. Gross N mineralization and ammonium consumption tended to increase in those soils associated with *L. palustre* compared to control soils, although plant presence only affected significantly gross N mineralization and leachate addition only affected ammonium consumption. Changes in mineralization were entirely explained by changes in ammonium concentrations since nitrate pools were always low. In the organic horizon, *L. palustre* presence as well as leachate addition produced no detectable changes on net N mineralization and microbial respiration probably because the variability within sites was very high. Since the chemical composition of organic soil C compounds (fibers, lignin, hemicellulose and cellulose), depth of organic horizon and understory biomass were similar between Ledum and non-Ledum sites, it is likely that the described effects on mineral soil are caused mainly by direct effects of *L. palustre* presence.

The higher ammonium immobilization and C mineralization and lower N-to-C mineralization suggest that addition of C compounds from *L. palustre* canopy and leachates stimulated microbial activity when microbes used them as a C substrate. Since a lower ratio between N and C mineralization is an indicator of the relative limitation of N to the microbial community (Schimel *et al.*, 1995), soils associated with *L. palustre* would enhance inorganic N immobilization in a N limited environment. Several studies have found that low molecular weight phenolic compounds can be readily metabolized by microbes, stimulating in this way soil respiration and microbial growth (Blum and Shafer, 1988; Boulfalis and Pellissier, 1994; Schimel *et al.*, 1995; Sparling *et al.*, 1981). In some cases the metabolization is as fast as with simple C compounds such as glucose (Sugai and Schimel, 1993). Our results partially support the readily metabolization of compounds present in leachates since soil respiration increased only the week after leachate addition. Increases in N immobilization induced by other C compounds released from the canopy such as carbohydrates (Magill and Aber, 2000; Michelsen *et al.*, 1995) or monoterpenes (Mackie and Wheatley, 1999; Vokou *et al.*, 1984)

cannot be discarded. However, leaching of water soluble compounds such as phenolics should exceed those components that are only slightly soluble in water, such as terpenes and lignin (Horner *et al.*, 1988).

Soils associated with *L. palustre* also increased gross N mineralization. This increase could be coupled with the use of C compounds as a substrate by microbes. Additions of C are expected to increase microbial biomass (Bradley *et al.*, 1997a) and thus to stimulate microbial turnover increasing gross mineralization as well as immobilization rates (Clein and Schimel, 1995).

The expected decreases on gross N mineralization and decomposition when condensed tannins form complexes with proteins, which has been identified as the major link between polyphenols and nutrient cycling (Hättenschwiler and Vitousek, 2000), were not found in our study. Although we cannot conclude that this process was not taking place, it was not relevant within the global effect of *L. palustre* presence and leachate addition. Proteins linked to phenolic compounds are likely to be less degraded by microbes than free organic N (Horner *et al.*, 1988; Palm and Sanchez, 1990) and this may lead to a conservation of N in the ecosystem (Northup *et al.*, 1998). These processes could be important in low N availability ecosystems with high soil C/N, such as boreal ecosystems. A conservative mechanism of N is not likely to happen in our system, since *L. palustre* presence and leachate addition accelerated N cycling.

L. palustre presence vs. leachate addition

Could the lower N availability in soils underneath *L. palustre* than in control soils be explained by the effects of soluble C compounds leached from litter? Since both plant presence and leachate addition increased C/N ratio and had similar effects on N transformation rates, our results suggest that litter leachates could be partially responsible for the plant presence effects. Although leachates released from *L. palustre* stimulate microbial activity for a few days, they may have longer-term effects on the nearby soil in natural conditions. Only a small percentage (5.9 % and 11.3% for non-Ledum and Ledum soils, respectively) of DOC added with the leachates was actually respired during the first week of incubation. If one third of the metabolized C was incorporated into microbial biomass while the rest was respired, as traditionally is assumed, and the increase in C mineralization of amended soils compared to control soils was a result of leachate addition, a 37 % and 41 % of DOC was readily metabolized by microbes within the first week. These percentages are very low when compared to those found by Sugai and Schimel (1993) who estimated that 90 % of two

phenolic acids were metabolized within 4 hours, and could indicate the presence of more recalcitrant compounds in *L. palustre* leachates which may be responsible for changes in N dynamics. Thus, a constant input of C compounds from *L. palustre* may not be required in order to change soil N cycling at long-term because those compounds not only affected short-term microbial activity but also changed soil nutrient quality by increasing C/N ratio. The low C mineralization could also due to organic matter retention by Al found at low pH. Changes in soil chemical properties after litter amendments, including increases in organic matter, total phenolics, K and PO_4, have also been found in *Ledum groenlandicum* (Inderjit and Mallik, 1997).

Negative interactions among plants through carbon-based secondary compounds: changes in N cycling or allelopathy?

Several arctic species have shown to inhibit plant growth, root elongation and seed germination of other species through the release of carbon-based secondary metabolites (Inderjit and Mallik, 1996b; Nilsson and Zackrisson, 1992). There is some controversy about whether those effects are caused by toxic effects of secondary metabolites, what is called allelopathy, or by indirect processes through changing soil nutrient availability, or both (Michelsen *et al.*, 1995; Wardle and Nilsson, 1997). Few studies have described the allelochemical mechanisms of action (Peñuelas *et al.*, 1996; Whitehead *et al.*, 1982). We found a lower net N mineralization and thus lower N availability in mineral soils under *L. palustre* canopy as a result of increasing microbial activity. Because organic horizon had negative net N mineralization, as is generally found in arctic soils during the growing season (Jonasson *et al.*, 1993), differences in mineral soils associated and non-associated to *L. palustre* could be important to determine plant N availability. This study, thus, supports the hypothesis that negative interactions among plants could be caused by changes in nutrient dynamics (Michelsen *et al.*, 1995; Schmidt *et al.*, 1997) although allelopathical effects can not be excluded. Changes in N cycling through the release of C compounds could be a general process for many species. However, not all studies found a lower N availability under plant canopy since more organic matter may accumulate (see Chapter 5). The dilemma of reduced plant growth mediated by secondary metabolites through chemical interactions among plants or through changes in soil fertility remains still open. Depending on the predominant processes in natural ecosystems, the ecological and evolutionary consequences would be rather different. Since allelopathy requires a species-specific coupled system of secondary metabolites and

their targets, this process could be less spread than changes in soil N availability through altering soil microbial activity. Ecologically, the latter could have a greater potential impact on the ecosystem because changing soil quality may at turn modify plant species establishment and successional dynamics (Clein and Schimel, 1995). Further research is needed to understand the importance of direct and indirect effects of plant species interactions in ecosystems.

Acknowledgments

The authors would like to thank Lola Oliver and Tim Quintal for their expert help in the laboratory. We also thank Josep Maria Alcañiz for critically reading this manuscript. E.C. has a predoctoral fellowship (FPI) from the Ministerio de Educacion y Cultura (Spain) in collaboration with Carburos Metalicos SA. We thank financial support from CICYT grants CLI99-0479 and REN-2000-0278/CLI (Spanish Government), IMMPACTE grant (DURSI and DMA, Catalan Government) and Fundació Territori i Paisatge (Catalonia).

Annex

		$N\text{-}NH_4^+$		$N\text{-}NO_3^-$	
		Initial	Final	Initial	Final
SITE (PLANT PRESENCE)	TREATMENT				
Organic soil					
Control	-	62.59 ± 2.84	48.79 ± 9.06	0.02 ± 0.01	0.43 ± 0.20
	+	62.59 ± 2.84	55.73 ± 14.28	0.02 ± 0.01	0.25 ± 0.15
Under *L. palustre*	-	43.94 ± 3.85	33.82 ± 14.16	0.03 ± 0.01	0.10 ± 0.05
	+	43.94 ± 3.85	27.52 ± 8.48	0.03 ± 0.01	0.11 ± 0.05
Mineral soil					
Control	-	5.10 ± 1.02	7.92 ± 1.80	0.01 ± 0.01	0.05 ± 0.03
	+	5.10 ± 1.02	4.82 ± 1.33	0.01 ± 0.01	0.02 ± 0.01
Under *L. palustre*	-	3.31 ± 0.98	4.06 ± 1.36	0.01 ± 0.00	0.03 ± 0.03
	+	3.31 ± 0.98	2.31 ± 0.79	0.01 ± 0.00	0.01 ± 0.01

Table I. Ammonium and nitrate concentrations (μg N g^{-1}DM) before and after a 28 days incubation of organic and mineral soils sampled under *L. palustre* and in a nearby control site, and amended with distilled water (-) or leachate (+).

Capítol 5

5. Is there a feedback between soil N availability in siliceous and calcareous soils and *Cistus albidus* leaf chemical composition?

E. CASTELLS, J. PEÑUELAS

Unitat d'Ecofisiologia CSIC-CREAF, Centre de Recerca Ecològica i Aplicacions Forestals (CREAF), Facultat de Ciències, Universitat Autònoma, Bellaterra 08193 (Barcelona), Spain

(Sotmès a publicació)

5.1. Abstract

The effects of the phenolic compound bearing shrub *Cistus albidus* on N cycling were studied in two siliceous (granitic-derived and schistic-derived) and one calcareous-derived soil differentiated by their acidity and texture. We aimed to know whether soils under *C. albidus* were affected by the release of C compounds from the canopy, and whether phenolic compound production in *C. albidus* changed depending on the soil N availability. Calcareous soils, with higher clay content and polyvalent cations, had a higher organic matter content but lower net N mineralization rates than siliceous soils, and *C. albidus* growing therein were characterized by lower foliar N and phenolic compound concentrations. Under *C. albidus*, all type of soils had higher phenolic compound concentrations and polyphenol oxidase activity. *C. albidus* presence and leachate addition decreased net N mineralization and increased soil respiration in siliceous soils, and this changes were related to a higher soil C/N ratio under the canopy. In calcareous soils, however, no significant effects of plant presence were found. In the studied plant-soil system it is not likely that higher phenolic compound concentrations were selected during evolution to enhance nutrient conservation in soil because (i) higher phenolic compound concentrations were not associated to lower soil fertilities, (ii) C compounds released from *C. albidus* accelerated N cycling and no evidences were found for decreases in gross N mineralization, and (iii) N retention in soil were more related to soil chemical and physical properties than to the effects of *C. albidus* canopy.

5.2. Introduction

Plant canopy presence can be a major determinant of small-scale patterns of nutrient availability by changing organic inputs and soil microbial activity (Aguilera *et al.*, 1999; Hook *et al.*, 1991; Inderjit and Mallik, 1999; Vinton and Burke, 1995). One of the ways plant presence can influence soil N dynamics is through rainfall leachates of phenolic compounds from green leaves and decomposing litter. Phenolic compounds may play a dominant role in controlling many aspects of plant-soil interactions, including nutrient availability and cycling, and organic matter dynamics by either increasing or decreasing microbial activity (Kuiters, 1990; Northup *et al.*, 1998; Schimel *et al.*, 1995). Phenolic compounds have been shown to decrease soil N availability through several mechanisms. First, they can form complexes with proteins, thus delaying organic matter decomposition and mineralization (Hättenschwiler and Vitousek, 2000; Horner *et al.*, 1988; Nicolai, 1988; Palm and Sanchez, 1990). Second, they can increase microbial activity and N immobilization (Blum, 1998; Blum and Shafer, 1988; Schimel *et al.*, 1995; Shafer and Blum, 1991; Sparling *et al.*, 1981; Sugai and Schimel, 1993). Third, they can directly inhibit fungal respiration (Boulfalis and Pellissier, 1994) and nitrification (Rice, 1984). The presence and relative importance of each one of those processes depend on the plant species and the molecular size, structure and concentrations of phenolic compounds (Hättenschwiler and Vitousek, 2000; Horner *et al.*, 1988; Kuiters, 1990; Schimel *et al.*, 1995).

Soil chemical and physical properties, such as pH, clay content and nutrient status, can also play an important role in the fate of phenolic compounds. Phenolic compounds require oxidation for most of their ecological activities, which varies with the physicochemical conditions of the environment such as soil redox (pe and pH) (Appel, 1993). Soil pH also determines the type and stability of bonds between phenolic compounds and organic matter, which likely affects their lability. Thus, at pH > 8 phenolic compounds can form irreversible covalent bonds with organic matter while at lower pH they tend to form hydrogen bonds which are characterized by their reversibility and lower strength (Appel, 1993). Presence of Ca can reduce the reactivity of phenolic compound functional groups by mediating the formation of bonds between clays and organic matter (Oades, 1988). On another hand, activity of polyphenol oxidases (PPO), a family of enzymes that participates in phenolic compounds degradation, has been also shown to increase with soil pH (Bending and Read, 1995; Pind *et al.*, 1994) and decrease with high clay content and cation-exchange capacity (Claus and Filip, 1990). Biological degradation rates of phenolic compounds can be influenced by nutrient

availability. When populations of microorganisms are nutrient limited the use of phenolic compounds as a C-source is restricted (Blum and Shafer, 1988).

There are also evidences that soil nutrient availability affects production of phenolic compounds. Resource availability hypotheses, such as Carbon-Nutrient Balance or Growth-Differentiation Balance (Bryant *et al.*, 1983; Herms and Mattson, 1992) predict increases in carbon-based secondary compounds (CBSC) concentrations, such as phenolic compounds, under high C-to-N availability. Changes in C source-sink relationships, as a consequence of reducing nutrient availability or increasing C availability, determine that plant growth is more restricted than photosynthesis. The resulting excess C is accumulated in total non-structural carbohydrates (TNC) and therefore synthesis of CBSC is enhanced (Peñuelas and Estiarte, 1998).

One of the most interesting hypotheses on the reciprocal interactions between plant phenolic compounds and soil nutrient cycling was proposed by Northup *et al.* (1998). Phenolic compounds would exert a conservative function of nutrients in a strongly acidic N-limited soil by binding proteins and delaying decomposition and mineralization and thus slowing N losses. If this process enhances conservation in the soil system, higher phenolic compound concentrations would be selected during evolution in those plants growing on a N-limited soil compared to plants with no nutrient restriction (Bending and Read, 1996; Northup *et al.*, 1995, 1998). The ecological relevance of phenolic compounds can be especially interesting in ecosystems such as Mediterranean ecosystems, where slow growing, N-limited species with high CBSC concentrations predominate. Moreover, in Mediterranean ecosystems, there is high spatial variability in soil nutrient availability since both siliceous and calcareous bedrock types, with strong different soil fertilities, dominate surface geology.

In order to study the reciprocal interactions between plant soluble phenolic compounds and soil N cycling dynamics, we selected a species (*Cistus albidus*) that has a high potential to release phenolic compounds by rainfall and decomposing litter, and can be found in either siliceous or calcareous soils. We analyzed N dynamics in mineral soils under *C. albidus* and in nearby control sites in three soils with different pH, carbonate content and texture (granitic, schistic and calcareous-derived soils) under similar conditions of precipitation, temperature and radiation in a Mediterranean area in NE Spain. The soils were also amended with leachates of *C. albidus* leaves and incubated in controlled conditions. We aimed to answer (I) whether presence of *C. albidus* individual plants, as well as leachate addition, changed N cycling dynamics and N availability, and whether those changes were affected by soil type, and (II) whether *C. albidus* phenolic compound concentrations changed depending on the soil

N status. We therefore wanted to answer whether a positive feedback between plant phenolic compound production and N availability can be found in our Mediterranean plant-soil system. Our hypothesis was that plants with higher phenolic compound production would grow on more infertile soils and phenolic compounds would stabilize organic matter decreasing mineralization rates and thus avoiding loses of N from the system.

5.3. Materials and methods

Sites description

The study sites were located in Capafons (Prades Mountains, SW of Barcelona, Spain) where different lythologies converge. The mean annual precipitation of this region is about 614 mm and the mean annual temperature is 13.9 ºC. We selected three different lythologies (granodioritic – granitic from now on for the sake of simplicity -, schistic and calcareous-derived soils) that differ in their physical and chemical properties (Table 9). In brief, siliceous soils (granite- and schist-derived soils) had lower pH, electric conductivity and carbonate content compared to calcareous soil. Organic matter, organic N, P and K^+ were also lower in siliceous soils. The three soils had a decreasing presence of sands and an increasing presence of clays from granitic to calcareous soils, with schistic soils having intermediate texture properties. To minimize differences in temperature and precipitation, all sites were established within 600 m of one another at the same elevation (ca 800 m) on a S or SW aspects. Vegetation from calcareous soil was naturally burned in summer 1994. The vegetation community was a low open shrubland with *Cistus albidus* and *Quercus ilex* as dominant species. Other species present in granitic soil were *Cistus salviifolius*, *Corylus avellana*, *Rosmarinus officinalis*, *Cistus laurifolius*, *Ulex parviflorus* and *Lavandula sp.;* in schistic soil: *Pinus halepensis*, *Corylus avellana*, *Vitis vinifera*, *Helichisum stoechas* and *Euphorbia characias*; and in calcareous soil: *Pinus halepensis*, *Quercus coccifera*, *Ulex parviflorus*, *Rosmarinus officinalis*, *Juniperus oxycedrus*, *Erica multifora*, *Dorycnium pentaphyllum*, *Daphne gnidium* and *Brachypodium retusum*.

	GRANODIORITE		SCHIST			CALCAREOUS		
Horizons	*A*	*B*	*A*	*B*	*C*	*A1*	*A2*	*B*
Maximum Depth (cm)	9-10	18-20	14-19	56-59	100	17	33	68
pH	7.1	7.2	6.3	7.0	7.2	7.8	8.0	8.3
Electrical conductivity (dS m^{-1})	0.08	0.04	0.12	0.07	0.11	0.28	0.25	0.17
$CaCO_3$ (%)	1	1	1	1	1	5	5	5
Organic matter (%)	2.0	0.8	2.5	0.9	0.5	10.2	4.2	2.3
Organic N (%) (Kjeldahl)	0.11	0.09	0.04	0.04	0.03	0.39	0.20	0.12
[P] (Olsen) (ppm)	5	1	6	2	3	8	2	1
[K+] (ppm)	132	43	398	152	81	273	154	131
Sand (% of fine earth)	92.5	95.1	58.3	62.6	77.0	36.9	36.4	39.8
Silt (% of fine earth)	4.8	3.1	31.8	26.6	15.1	38.9	38.4	41.3
Clay (% of fine earth)	2.7	1.8	9.9	10.8	7.9	24.2	25.2	18.9
USDA classification	sand	sand	sandy loam	sandy loam	sandy loam	loam	loam	loam

Table 9. Physical and chemical properties of the substrates selected for this study.

Studied species

Cistus albidus is an evergreen shrub up to 1m high with a leaf longevity not longer than one year, and frequently found in xeric or disturbed sites. This species can be found both in siliceous and in calcareous soils and preliminary analyses showed that it can release high concentrations of phenolic compounds from green leaves and litter. It also has a high density of hairs and glands both on green shoots and on leaf surfaces which exudate phenolic compounds (Fig. 19).

Plant and Soil sampling

Two different samplings (winter and summer) were conducted for soils and plants. Since plant phenolic compound concentrations have been shown to vary depending on the season (Feeny 1970), changes in precipitation along the year (with maximum at spring and autumn in Mediterranean climate) can affect the amount of soluble C compounds released from the plant. The temporal variability in the input of C compounds to the soil could affect nutrient dynamics. Three and four sites (sampled during March and July 2000, respectively) were located within each type of soil at ca 20 m of each one. In each site, we selected five *C. albidus* plants that formed independent units and five nearby control plots. Control plots were

located close to each selected *C. albidus* plant at the same elevation or slightly higher in order to avoid effect of green leaves or litter leachates released by rainfall. In the granitic and calcareous soils no herbaceous vegetation was present among *C. albidus* shrubs. Under-plant soil samples were taken just under *C. albidus* on the downslope side of the stem where litter was present. Soils were sampled at 15 cm depth using a 5 cm diameter core. Two cores, one in control soil and one underneath *C. albidus* were sampled in each plot (10 samples in total) and bulked together within site. Soils were sieved with a 2 mm mesh and kept at 4 ºC until the experiments started.

C. albidus leaves were sampled in each one of the 5 selected plants within site (15 plant samples per soil type in March and 20 in July). They were dried at 65 ºC for 48 h. Standing leaf litter was also sampled from the same plants during June 2000.

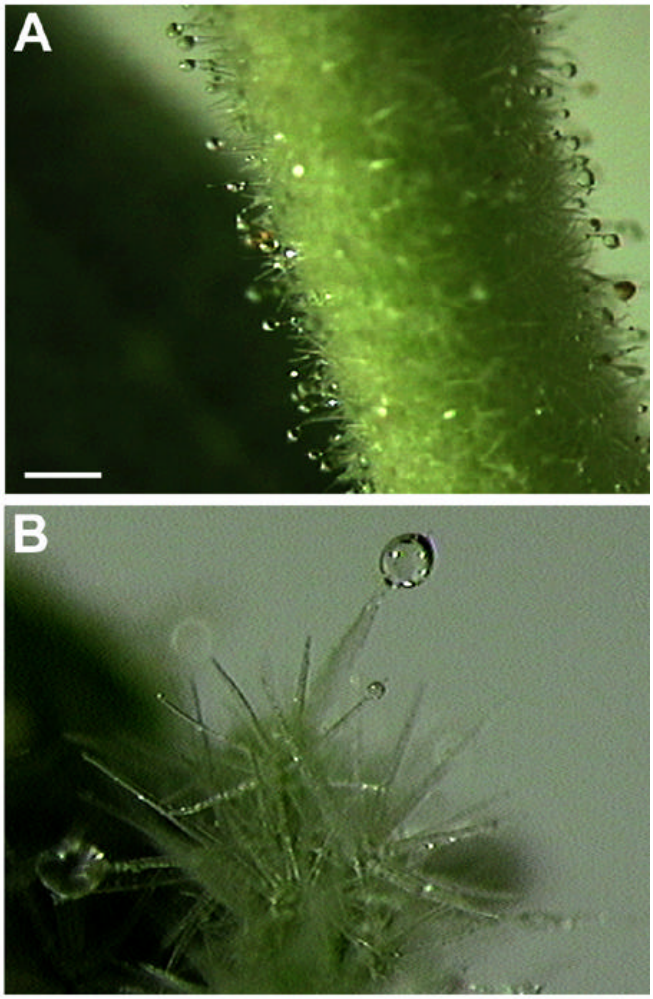

Figure 19. (A) *C. albidus* petiole glands containing phenolic compounds. High density of glands and trichomes are also found in both surfaces of green leaves and leaf litter. (B) detail of glands and trichomes. Scale bar in A: 1 mm.

Biomass of *C. albidus* plants was estimated by measuring the basal diameter of all shoots per each of the 60 selected plants and their average height. Thereafter, the shoot volume was calculated considering each shoot as a cone shape. Mastrantonio (2000) found that the sum of shoot volumes within an individual plant of *C. albidus* was a good allometric estimator for plant biomass (r^2= 0.89). Plant area was calculated by measuring the canopy maximum length and its perpendicular axis. Canopy shape was considered an ellipse with a radium equal to half of the canopy lengths. We estimated plant biomass per soil area dividing the resulting estimation of plant biomass by the canopy area. *C. albidus* plants growing on granitic and schistic soils had higher biomass and occupied larger area than plants growing on calcareous soil (p = 0.002, t-test). However, plant biomass per soil area was homogeneous among soil soils (p = 0.93, t-test).

Plant analyses

Leaf total phenolic compound concentrations from green leaves and leaf litter were determined by Folin-Ciocalteu method improved by using a blank of polyvinylpolypyrrolidone (PVPP) (Marigo, 1973). PVPP removes phenolic substances from the solution and avoids an overestimation of total phenolic compounds due to non-phenolic Folin-Ciocalteu reactive substances. Gallic acid was used as standard. Condensed tannins were analyzed by proantocyanidin method (Waterman and Mole, 1994) and cyanidine chloride was used as a standard.

The leaf $\delta^{15}N$ and $\delta^{13}C$ ratios were measured on sub-samples (about 1 mg) of ground leaves by a dual inlet triple collector isotope ratio mass spectrometer (SIRA II, VG Isotech, Middlewich, UK), operated in direct inlet continuous flow mode. Before analysis, the solid samples were combusted in a Dumas-combustion elemental analyzer (Model NA 1500, Carlo Erba, Milano, Italy) for organic C and organic N analyses. The reference CO_2, calibrated against Pee Dee Belemnite standard (PDB), was obtained from Oztech (Dallas, TX, USA). The analytical precision of the measurement was ± 0.2 ‰ for $\delta^{15}N$ and ± 0.1 ‰ for $\delta^{13}C$.

Leachates preparation

A *C. albidus* leachate was obtained by shaking fresh green leaves (25 g equivalent DM), collected from schist-derived soils, in 1 L of distilled water for 24 h at room temperature (Zackrisson and Nilsson, 1992). The resulting leachate was filtered through a filter paper

Whatman 42 and analyzed for total phenolic compounds, condensed tannins (see methods above) and dissolved organic carbon (DOC) (Shimadzu TOC-5000). We also measured phenolic compound and condensed tannin concentrations in *C. albidus* leaves before and after making the leachate. A fractionation of leachates into non-phenolic and phenolic fractions using a C-18 solid-phase extraction column (Extra-sep, LIDA) was analyzed for DOC in order to know what percentage of leachate DOC was contributed by phenolic compounds.

Soil incubations

Soils sampled under *C. albidus* and in the nearby control sites were incubated for 28 days. Mineral soil (30 g) was placed in glass jars fitted with septa for taking headspace samples, and 3.5 mL of *C. albidus* leachate or distilled water (control) was added to the soils just before starting the incubation. Field capacity was measured from a soil subsample using a modified procedure from Tan (1996). Additional distilled water was added to the soils until field capacity when necessary, and they were incubated at 25 ºC. A soil subsample per type of soil (15 g FW) were extracted with 75 mL 2 M KCl for 1 h and analyzed for initial ammonium and nitrate by an auto-analyzer (Flow Injection Analyzes, FOSS, Höganäs, Sweden). The C mineralization was estimated by analyzing CO_2 accumulated in jars over a 4 to 7 days period by a gas chromatograph provided with a Porapaq QS column and a thermal conductivity detector (Hewlett Packard 5890 Series II). After each measurement, jars were opened and ventilated in order to reestablish ambient CO_2 concentrations, and distilled water was added when necessary to maintain field capacity. After 28 days, final ammonium and nitrate concentrations were measured. The net N mineralization rates were calculated subtracting initial concentrations from final concentrations, and were expressed per unit of soil dry weight and per unit of organic C. The C mineralization was calculated from the total CO_2 produced during the incubation.

Soil analyses

A ground soil subsample was analyzed for organic C and N with an elemental analyser (Model NA 1500, Carlo Erba, Milano, Italy) using the standard configuration for those determination. Organic C in calcareous soils was analyzed by the Walkley-Black procedure from the rapid dichromate oxidation techniques (Nelson and Sommers, 1982). Soil pH and potential pH were analyzed after shaking fresh soils with distilled water or 0.1 M KCl (1:2.5 w/v) for 30 min.

The phenolic compounds of the soil were extracted with NaOH following Whitehead *et al.* (1982). Alkaline extractions are normally used to remove phenolic compounds reversibly bound to the soil. Twenty grams of soil was placed in a test tub with 50 mL NaOH 2 M (1:2 w/v) under N_2 and shaked for 20 h at room temperature followed by filtration through Whatman 44 filter paper. Concentrations of phenolic compounds were determined using Folin-Ciocalteu method described earlier.

The polyphenol oxidase (PPO) activity was assessed using the method of Nichols-Orians (1991), which determines the conversion of catechol to *p*-quinone, a compound that absorbs light at 420 nm. PPO participates in the degradation of phenolic compounds by breaking bonds between phenolic compounds and other organic compounds. Enzyme was extracted from soil by shaking the fresh soil with 50 mM of cold acetic acid-acetate buffer at pH 5 (1:6 w/v) during 30 min, centrifuging for 5 min at 4200 rpm and filtering through Whatman GF/A glass fiber filter. 500 µL of the resulting extract were added to 500 µL of 0.1 M citrate-phosphate buffer, 100 µL of distilled H_2O and 100 µL of 3% (w/v) catechol. As PPO activity varies with pH (Bending and Read, 1995; Leake and Read, 1990), we conducted the analyses using citrate-phosphate buffer pH at 6, 7 and 8, choosing conditions similar to the studied pH soil soils. After mixing, the absorbance of the solution was measured at 420 nm. Tubes were placed in a 25 ºC water bath and after 1 h absorbance at 420 nm was remeasured. The PPO activity was calculated by subtracting the initial absorbance to the final absorbance. Results were expressed as absorbance units (AU) per g^{-1} soil DM h^{-1}.

Statistical analyses

A two-way ANOVA, one-trial repeated measures ANOVA or two-trial repeated measures ANOVA were conducted depending on the experiment (see table legends for more details). No transformations were needed in order to fit a normal distribution in any case. An estimate of plant density was used as a covariant in the soil experiments. Analyses were performed using Statistica 99 (Statsoft, Inc; Tulsa, USA).

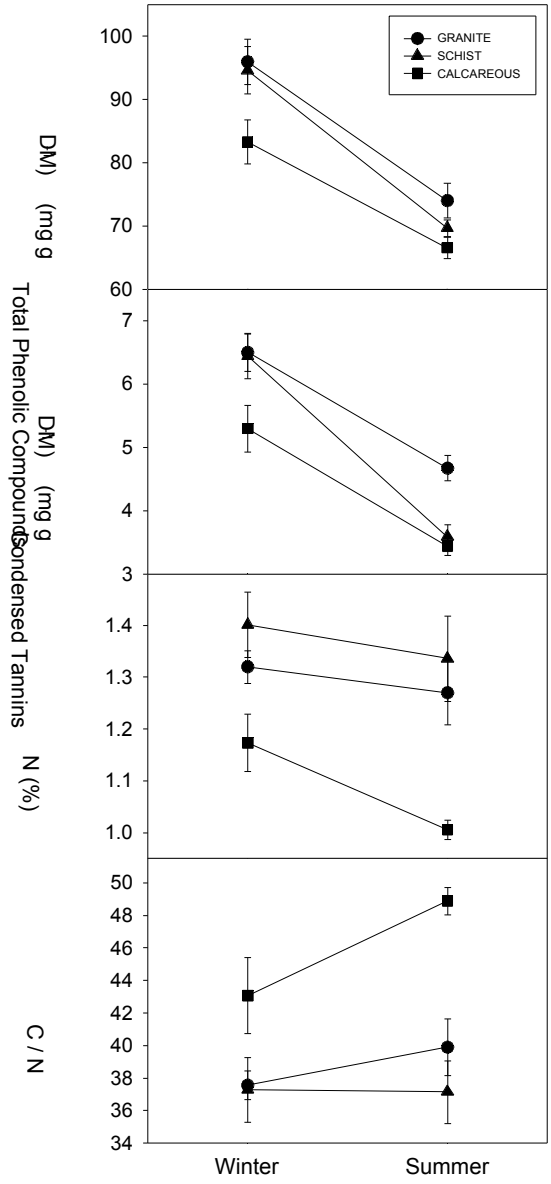

Figure 20. Phenolic compounds, condensed tannins, N and C/N ratio from leaves of *C. albidus* growing on granitic, schistic or calcareous soils. Values represent means and SE (n = 15 for phenolic compounds and condensed tannins in winter samples and n = 20 for summer samples; n = 9 for N and C/N in winter and summer samplings). Statistical analyses are shown in Table 10.

5.4. Results

Plant analyses

Phenolic compound concentrations in *C. albidus* green leaves ranged from 66.5 to 95.9 mg gallic acid g^{-1} DM. They varied during the year and with soil type, with maximum phenolic compound concentrations in winter and in plants growing on granitic and schistic soils (Fig. 20, Table 10). Litter phenolic compounds sampled during winter ranged from 27.0 mg gallic

acid g^{-1} DM in calcareous soil to 56.7 mg gallic acid g^{-1} DM in granitic soil, following the same pattern than in green leaves, with higher concentrations in granitic and schistic compared to calcareous soil, but with lower values compared to green leaves. Condensed tannins and leaf N concentrations had higher values in plants growing on granitic and schistic soils and in winter leaves (Fig. 20, Table 10). There was a significant interaction between soil type and sampling time for leaf N concentrations, with a stronger decrease in summer plants growing on calcareous soils than those on other soils. Leaf C/N ratio was lower in plants growing on granitic and schistic soils and in leaves sampled during summer (Fig 20, Table 10). Post-hoc comparisons for soil type showed that plants growing on granitic and schistic soils were significantly different from those of plants growing on calcareous soil for phenolic compounds, condensed tannins, N concentrations and C/N ratio (Table 10).

Plant stable isotopes analyses showed differences in C and N fractionations depending on date of sampling, with more negative $\delta^{13}C$ and $\delta^{15}N$ in leaves sampled during summer. $\delta^{15}N$ also changed depending on the soil type, being more negative in plants growing on granitic soil (Fig. 21, Table 10)

C. albidus leachate had 329.6 mg L^{-1} of total phenolic compounds, 67.2 mg L^{-1} of condensed tannins and 1190 mg L^{-1} of DOC. Total phenolic compounds comprised 46 % of the total DOC present in the leachate. The leachate removed a 33.7 % of the phenolic compound concentrations and 29.2 % of the condensed tannin concentrations present in *C. albidus* leaves.

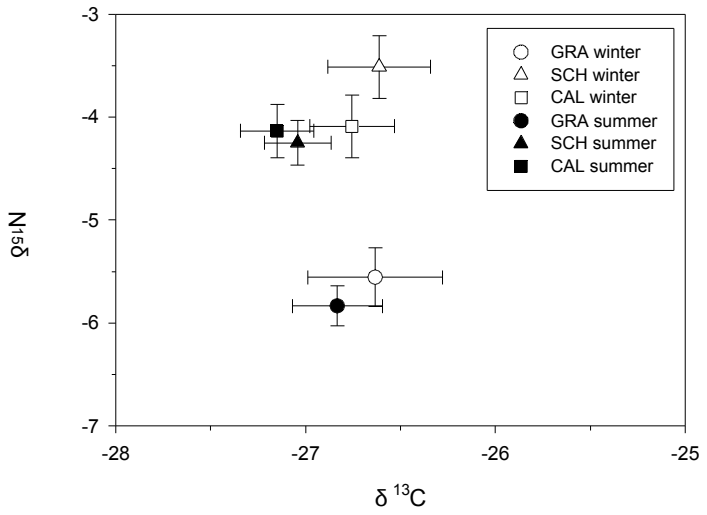

Figure 21. $\delta^{13}C$ versus $\delta^{15}N$ from *C. albidus* growing on granitic (GRA), schistic (SCH) or calcareous (CAL) soil. Values represent means and SE (n = 9). Statistical analyses are shown in Table 10.

	Phenolic Compounds		Condensed Tannins		N		C/N		$\delta^{13}C$		$\delta^{15}N$	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
SOIL	3.86	**0.029**	6.56	**0.003**	10.80	**<0.001**	11.28	**<0.001**	0.005	0.99	3.86	**<0.001**
SEASON	28.39	**<0.001**	125.3	**<0.001**	7.18	**0.013**	4.38	**0.046**	3.55	0.071	4.13	0.053
SOIL x SEASON	0.37	0.82	1.66	0.2	2.84	0.07	2.09	0.14	0.16	0.84	2.41	0.11
GRA – SCH		0.99		0.45		0.99		0.98		0.99		**<0.001**
GRA – CAL		0.062		**0.002**		**0.001**		**0.001**		0.99		**<0.001**
SCH – CAL		**0.044**		**0.05**		**0.001**		**0.001**		0.99		0.78

Table 10. Repeated measures ANOVA for leaf total phenolic compounds, condensed tannins, N, C/N and isotope discrimination for ^{13}C and ^{15}N from *C. albidus* plants sampled during winter and summer (SEASON) and growing on granite - GRA - , schist - SCH - and calcareous - CAL- soils (SOIL). (n = 15 for total phenolics and condensed tannins, and n = 9 for the other variables). Post-hoc comparisons for SOIL were conducted using Tukey HSD test. $p<0.05$ are highlighted in bold.

		pH (H_2O)		pH (0.1 M KCl)		Phenolic Compounds (mg Gallic acid g^{-1}DM)		PPO activity ($AU\ g^{-1}DM\ h^{-1}$ x 1000)	
GRANITE	Control	6.7		5.6		0.43		0.99	
	Under plant	6.9		6.1		0.62		2.49	
SCHIST	Control	6.3		5.5		0.87		2.10	
	Under plant	6.3		5.8		0.93		2.77	
CALCAREOUS	Control	7.9		7.4		1.25		6.05	
	Under plant	8.0		7.5		1.34		6.55	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
SOIL		71.91	**<0.001**	99.98	**<0.001**	37.30	**<0.001**	39.14	**<0.001**
PLANT		1.33	0.27	6.06	**0.03**	5.18	**0.048**	10.33	**0.012**
SOIL x PLANT		1.24	0.33	3.22	0.08	0.56	0.58	0.91	0.43
GRANITE – SCHIST			**0.011**		0.17		**0.007**		0.32
GRANITE – CALCAREOUS			**<0.001**		**<0.001**		**<0.001**		**<0.001**
SCHIST – CALCAREOUS			**<0.001**		**<0.001**		**0.005**		**0.001**

Table 11. Mean values and statistical analyses for soil pH, potential pH, phenolic compound concentrations and polyphenol oxidase (PPO) activity for soils sampled under *C. albidus* and a nearby control site (PLANT) at granite, schist and calcareous soils (SOIL). A repeated measures ANOVA (PLANT) was conducted using estimated plant biomass per soil area as a covariant (n = 15). Post-hoc comparisons for SOIL were conducted using Tukey HSD test. $p<0.05$ are highlighted in bold.

Soil Analyses

Soils sampled under *C. albidus* had higher potential pH (KCl) than control soils, and differences were stronger in granitic soil (Table 11). Concentrations of both soil organic

matter and reversibly bound phenolic compounds were lower in granitic soils and higher in calcareous soils. Within soil type they were always higher in soils sampled under *C. albidus* than in soils from nearby control sites (Table 11). PPO showed a stronger activity measured at pH 6 in calcareous soils and in soils sampled under *C. albidus*. No PPO activity was found at pH 7 or 8 in any soil type (Table 11).

Organic C and N concentrations differed depending on the soil type and date of sampling, with higher values in calcareous soils and in winter (Fig. 22, Table 12). Organic C and N concentrations in all soils were higher under *C. albidus*. A significant interaction was found for both variables between soil type and sampling date, since calcareous soils had a stronger decrease in organic C and N during summer compared to siliceous soils (Table 12). Calcareous soils had a higher C/N ratio than siliceous soils. The significant interaction between soil type and plant presence indicated that whereas C/N ratio was higher under C. albidus in siliceous soils, no changes due to plant presence were found in calcareous soils (Fig 22, Table 12).

Soil type, plant presence and sampling time had also an effect on soil microbial activity. Thus, potential net N mineralization expressed on a weight basis was higher in schistic soils than in granitic and calcareous soils, and lower under *C. albidus* plants and in winter. Leachate addition had no effects on net N mineralization expressed per gram soil (Table 13). However, net N mineralization per unit C was higher in granitic and schistic compared to calcareous soil and it changed depending on plant presence and sampling date. For the summer and winter samplings *C. albidus* presence decreased N mineralization (g^{-1} C) in siliceous soils but no changes were found in calcareous soils. Plant presence effect on net N mineralization was not related to sampling date. Additions of plant leachate slightly decrease net N mineralization in all soils although statistical differences were marginally significant (Fig. 23, Table 13).

C mineralization in soils sampled in summer was higher for granitic and schistic soils and under *C. albidus* in all soils and incubation dates although the effect was stronger early in the incubation (Fig. 24 and 25, Table 13). Leachate addition also affected C mineralization the first two weeks of soil incubation, although no global effects were found (Fig. 25, Table 13). The ratio between net N mineralized and C mineralized, which indicate the relative limitation of this nutrients, was higher for granitic and schistic compared to calcareous soils. Under *C. albidus* this ratio was lower than in the respective control ground in siliceous soils but it was higher in calcareous soils. Leachate addition decreased it independently of the analyzed soils (Fig. 24, Table 13). Post-hoc comparisons showed different responses of calcareous soil

compared to granitic and schistic in net N mineralization per unit C, C mineralization and N-to-C mineralization (Table 13).

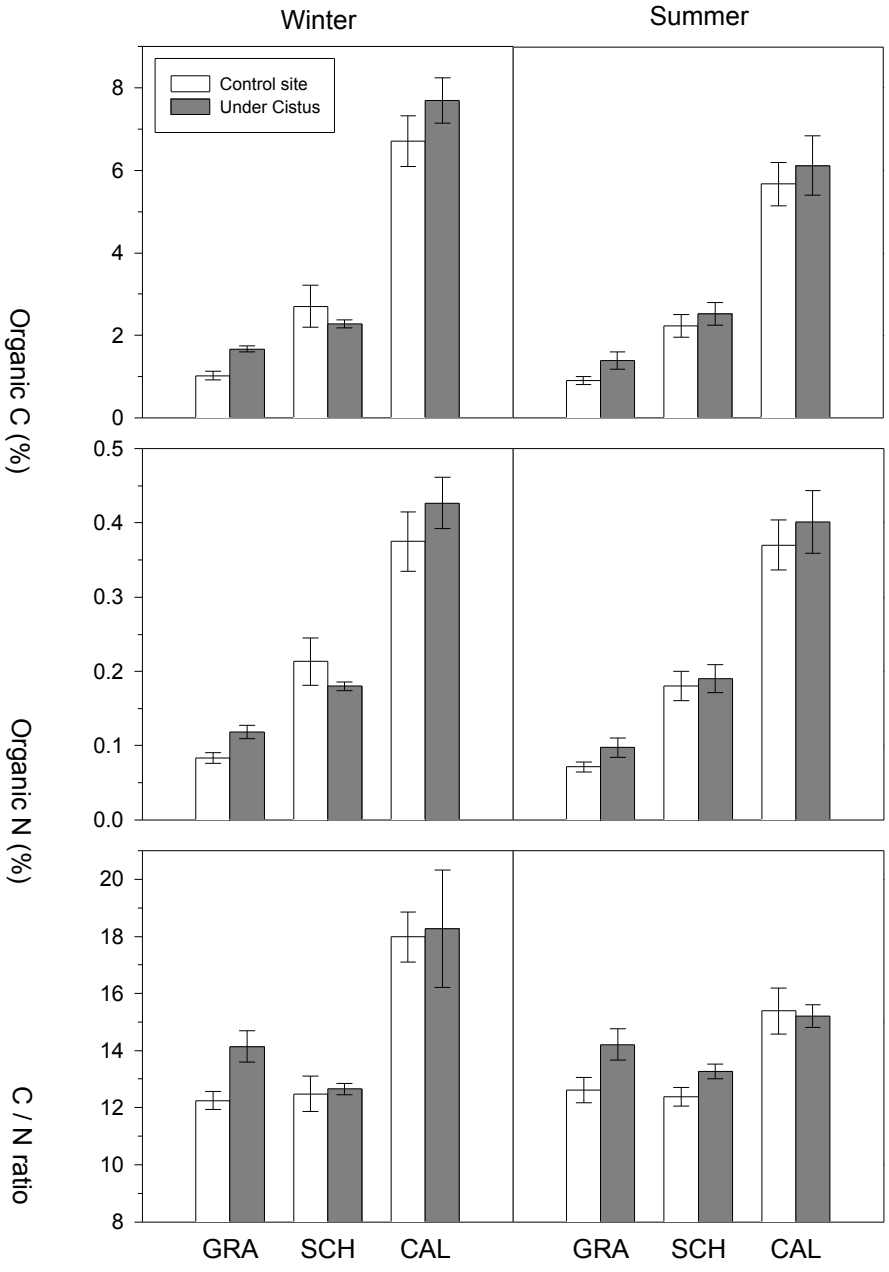

Figure 22. Organic C and N concentrations and C/N ratio from soils sampled in winter and summer under *C. albidus* and in a nearby control site at granitic (GRA), schistic (SCH) and calcareous (CAL) soils. Values represent means and SE (n = 4). Statistical analyses are shown in Table 12.

	Organic C		Organic N		C/N	
	F	*p*	*F*	*p*	*F*	*p*
SOIL	57.20	**<0.001**	28.68	**0.001**	63.75	**<0.001**
PLANT	7.36	**0.03**	4.25	0.08	19.61	**0.004**
SEASON	10.72	**0.016**	19.95	**0.004**	0.54	0.48
SOIL x PLANT	2.03	0.21	2.21	0.19	12.65	**0.007**
SOIL x SEASON	7.97	**0.02**	4.09	0.075	3.33	0.10
PLANT x SEASON	0.007	0.93	0.08	0.78	0.03	0.85
SOIL x PLANT x SEASON	0.75	0.50	1.68	0.26	0.44	0.65
GRANITE – SCHIST		0.13		0.1		0.25
GRANITE – CALCAREOUS		**<0.001**		**0.001**		**0.001**
SCHIST – CALCAREOUS		**0.001**		**0.01**		**<0.001**

Table 12. Statistical analyses for organic C, organic N and C/N ratio of soils sampled under *C. albidus* and in a nearby control site (PLANT) at granite, schist and calcareous substrates (SOIL) in winter and summer (SEASON). Two-trial repeated measures ANOVA (PLANT and SEASON) was conducted using estimated plant biomass per soil area as a covariant (n = 3). Post-hoc comparisons for SOIL were conducted using Tukey HSD test. p<0.05 are highlighted in bold.

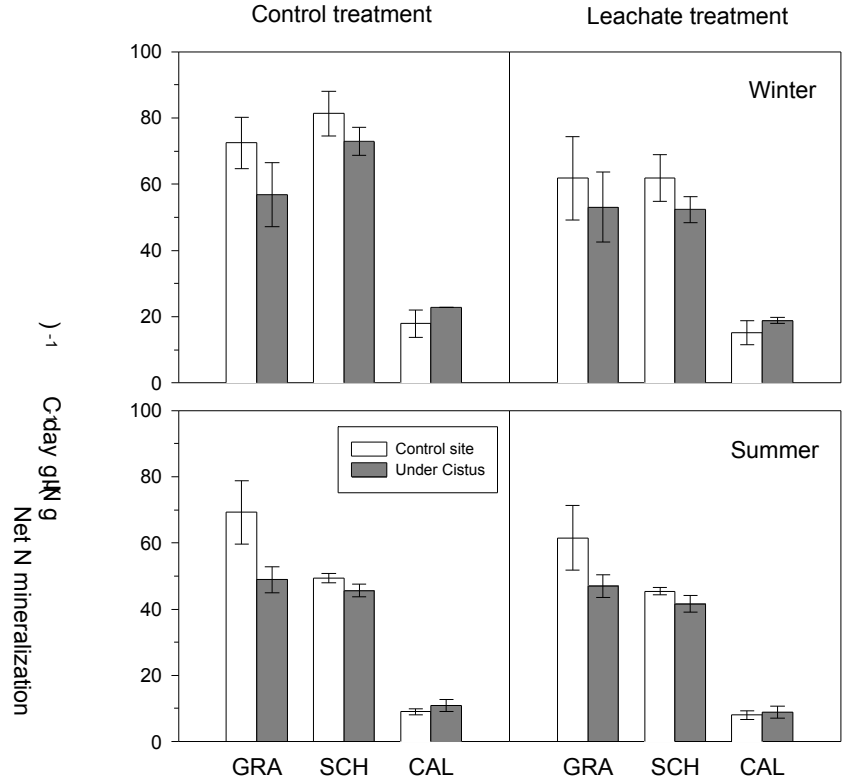

Figure 23. Net N mineralization rates per unit C of winter and summer soils sampled under *C. albidus* and in a nearby control site at granitic (GRA), schistic (SCH) and calcareous (CAL) soils. Soils were amended with leachate or distilled water (control treatment). Values represent means and SE (n = 4). Statistical analyses are shown in Table 13.

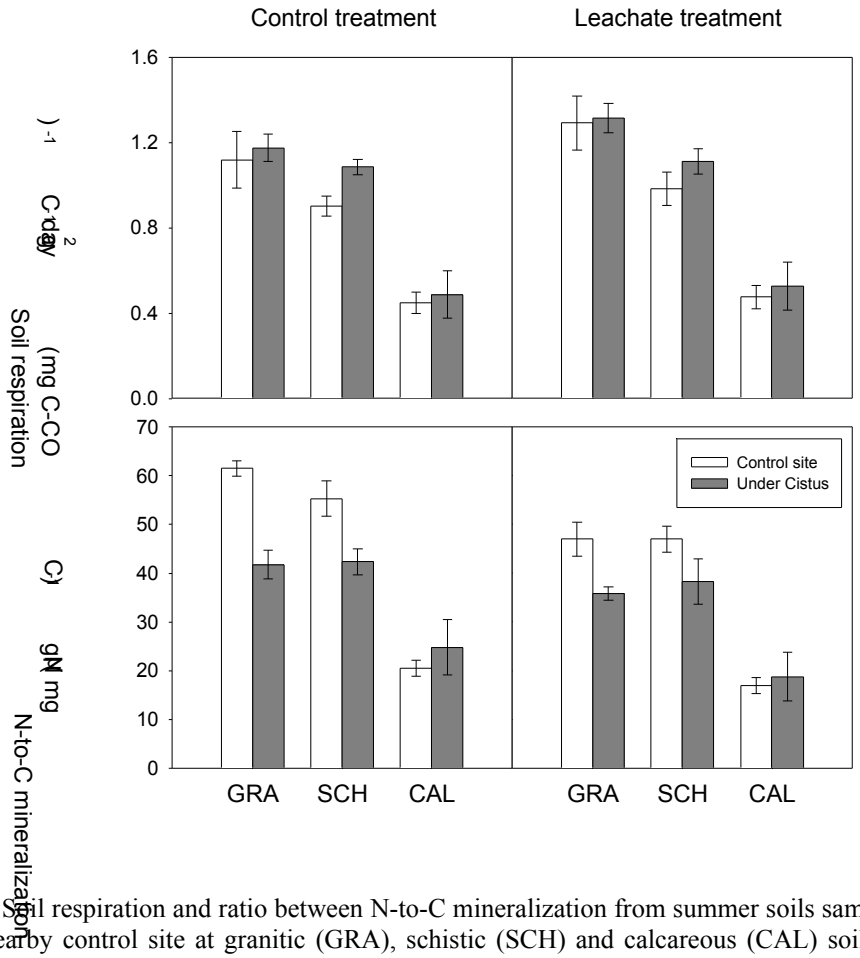

Figure 24. Soil respiration and ratio between N-to-C mineralization from summer soils sampled under *C. albidus* and in a nearby control site at granitic (GRA), schistic (SCH) and calcareous (CAL) soils, and amended with leachate or distilled water (control treatment). Values represent means and SE (n = 4). Statistical analyses are shown in Table 13.

5.5. Discussion

Differences in physical, chemical and biological properties among soils

The soils analyzed in our study were highly differentiated in their physical and chemical properties, as well as in their biological activity. Calcareous soils had higher total organic matter content than granitic and schistic soils. In a similar temperature and water regimes, soil organic matter level is affected by soil texture and presence of polyvalent cations (e.g. Ca^{2+}, Fe^{3+}) (Oades, 1988). High clay content is an important mechanism for stabilizing organic matter because clay particles tend to bind to organic matter and protect it from microbial degradation (Hassink, 1994; Oades, 1988; Parton *et al.*, 1987). As a result, rates of decomposition, mineralization or N immobilization can be slower and organic matter, specially the more recalcitrant one, can accumulate (Ladd *et al.*, 1977; Wardle, 1998). Our results fits in this discussion since calcareous soils (with higher clay and carbonate content

than siliceous soils) had lower microbial activity per organic carbon than siliceous soils. We should also consider that organic matter accumulated in calcareous soils may be more recalcitrant compared to that from siliceous soils and thus more difficult to decompose by microorganisms. However, not all N transformation processes were slowed proportionally since the C/N ratio and the ratio between N-to-C mineralization differed among soils. The N-to-C mineralization ratio is an indicator of the relative extent of N or C limitation to the soil microbial community (Schimel *et al.*, 1995). The presence of a lower ratio, such as in calcareous soils, indicate the presence of a N limited community that is either processing N poor material or immobilizing a large portion of the mineralized N, while a higher ratio indicates a C limited community that has excess N relative to its needs (Schimel *et al.*, 1998). Ammonium retention by clays in calcareous soils could also contribute to a lower N-to-C mineralization. Thus, these calcareous soils were relatively more N limited than siliceous soils, which determined a lower plant N availability and lower leaf N content in *C. albidus*. Moreover, N loses or outputs of N in calcareous soils were greater than in granitic soils as indicated by $\delta^{15}N$ data (Peñuelas *et al.*, 2000).

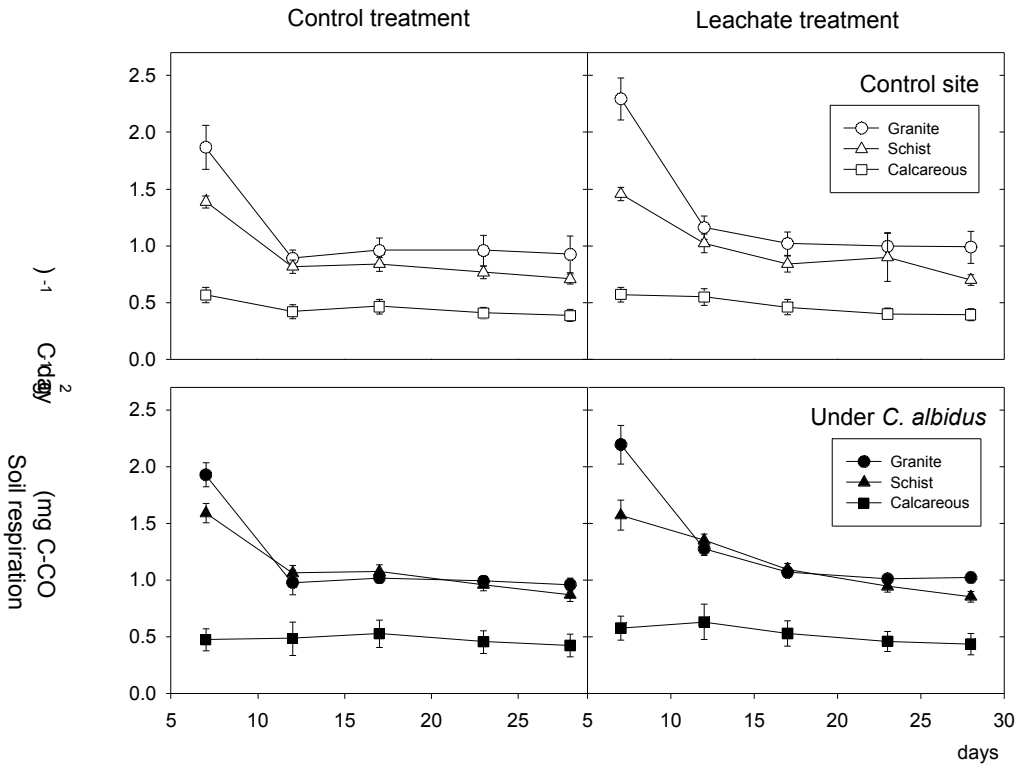

Figure 25. Respiration along a 4-week incubation of summer soils sampled under *C. albidus* (closed symbols) and in a nearby control site (open symbols) at granitic (GRA), schistic (SCH) and calcareous (CAL) soils. Soils were amended with leachate or distilled water (control treatment). Values represent means and SE (n = 4). Statistical analyses are shown in Table 13.

	Net N mineralization (g^{-1} DM)		Net N mineralization (g^{-1} C)		C mineralization (g^{-1} C)		N-to-C mineralization	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
SOIL	13.29	**0.001**	41.61	**<0.001**	38.58	**<0.001**	61.17	**<0.001**
TREATMENT	2.26	0.16	3.10	0.11	1.37	0.26	10.59	**0.005**
PLANT	0.8	0.39	20.39	**0.001**	2.50	0.14	20.02	**<0.001**
TIME	103.88	**<0.001**	29.18	**<0.001**	208.57	**<0.001**	-	-
SOIL x TREATMENT	0.39	0.68	0.51	0.61	0.40	0.67	0.67	0.52
SOIL x PLANT	5.6	**0.02**	7.33	**0.01**	2.80	0.10	10.16	**0.001**
TREATMENT x PLANT	0.02	0.87	0.20	0.66	0.01	0.91	0.82	0.37
SOIL x TIME	11.57	**0.002**	3.00	0.09	41.74	**<0.001**	-	-
TREATMENT x TIME	3.48	0.09	1.71	0.21	5.84	**<0.001**	-	-
PLANT x TIME	0.003	0.95	0.07	0.78	3.07	**0.02**	-	-
SOIL x TREATMENT x PLANT	0.11	0.89	1.07	0.37	0.08	0.91	0.98	0.39
SOIL x TREATMENT x TIME	1.04	0.38	0.79	0.47	1.65	0.13	-	-
SOIL x PLANT x TIME	4.01	**0.05**	0.14	0.86	0.35	0.93	-	-
TREATMENT x PLANT x TIME	0.01	0.9	0.01	0.90	0.79	0.53	-	-
SOIL x TREATMENT x PLANT x TIME	0.01	0.98	0.001	0.99	0.23	0.98	-	-
GRANITE – SCHIST		**0.001**		0.99		**0.03**		0.94
GRANITE – CALCALREOUS		0.3		**<0.001**		**<0.001**		**<0.001**
SCHIST – CALCAREOUS		0.06		**<0.001**		**<0.001**		**<0.001**

Table 13. Statistical analyses for net N mineralization expressed per unit DM and unit carbon, C mineralization and ratio between N to C mineralization for soils sampled under *C. albidus* and in a nearby control site (PLANT) at granite, schist and calcareous substrates (SOIL). Soils were amended with distilled water or with *C. albidus* leachate (TREATMENT). Two-way (SOIL and TREATMENT) two-trial repeated measures ANOVA (PLANT and TIME) were conducted for net N mineralization and CO_2 production. For net N mineralization, TIME variable is referring at winter and summer samplings, i.e. season, and for CO_2 production is referring at date of sampling along a one-month incubation period for soils sampled in summer. Ratio between net N to C mineralization was tested by a two-way ANOVA (SOIL and TREATMENT) with a repeated measures for PLANT. Estimated plant biomass per soil area was used as a covariant in all cases (n = 4). Post-hoc comparisons for SOIL were conducted using a Tukey HSD test. $p<0.05$ are highlighted in bold.

Plant presence effects on organic matter and N cycling

Plant presence affected soil N cycling independently from changes in *C. albidus* chemical composition. Thus, although leaf *C. albidus* phenolic compound and N concentrations and C/N ratio changed between winter and summer seasons, no differences between sampling date were found for microbial activity under *C. albidus*. Vinton and Burke (1995) also found that presence of a plant rather than the plant properties was more relevant in order to predict its effects on the soil system. In our study plant presence effects on soil were dependent on the soil type. Thus, in siliceous soils (granite and schist), a higher soil C/N ratio was found under *C. albidus*, and this changes were correlated with higher soil respiration and lower net N mineralization and N-to-C mineralization compared to the same processes in control soils. However, in calcareous soils, plant presence had no effect on soil C/N ratio or microbial activity. Differences between siliceous and calcareous soils may be determined by the

proportional inputs of organic matter from *C. albidus* compared to the accumulated organic matter under plants. While inputs of organic C and N from *C. albidus* represented a 54.4 % and 42.8 % from the total organic C and N in granitic soil, respectively, the percentage decreased up to 7.9 % and 8.1 % in calcareous soils since the organic matter pool in the latter was much higher. Thus, N dynamics in calcareous soils was less constrained by releases of organic matter from *C. albidus* than silicious soils.

Other explanations may account for the less responsive calcareous soils to *C. albidus* presence compared to siliceous soils. The effect of phenolic compounds released from the plant could be affected by soil nutrient status. When microorganisms populations are more N limited, such is the case of calcareous soils, they restrict the use of phenolic compounds as C-source (Blum and Shafer, 1988). Moreover, Ca present in calcareous soils could reduce the reactivity of the functional groups of phenolic compounds and other organic compounds by forming bounds with clays. Since both clays and organic matter (humus) are negatively charged, the polyvalent cations bridge organic molecules to clay particles forming aggregates which are physically, chemically and biologically stable (Oades, 1988). Moreover, at pH > 8 phenolic compounds tend to form irreversible covalent bonds with organic matter (Appel, 1993). Finally, soil organic matter retention in calcareous soil may be enhanced by the lower degradation of the complexes between phenolic compounds and proteins. The first stage of phenolic compound mineralization, and the consequent release of organic N, is mediated by polyphenol oxidases (PPO). This family of enzymes is synthesized by ectomycorrhizal fungi (Leake and Read, 1989) and has been shown to change with pH environments (Bending and Read, 1995) and clay-rich soils (Claus and Filip, 1990). Although in our study PPO activity at pH 6 was higher in calcareous soils, no activity was detected at pH 8, its actual pH in the field. Thus, calcareous soils may have lower phenolic compound degradation in field conditions compared to granitic or schistic soils.

The effects of *C. albidus* presence could be caused, at least partially, by C compounds leached from green leaves and litter. The addition of *C. albidus* leachate had a similar effect than plant presence, decreasing N mineralization and increasing C mineralization early in the incubation. The lower N-to-C mineralization ratio in amended soils showed that the most relevant process affecting N cycling was an increase of inorganic N immobilization when microbes used C compounds present in the leachates as a C source. However, additional changes in N cycling, such as decreases in gross N mineralization, cannot be excluded. A further analysis of gross N transformation rates is necessary in order to know what processes are exactly affecting both plant presence and leachate addition. Since phenolic compound

concentrations and PPO activity in soils under *C. albidus* were higher compared to control soils, leachate effects may be due to phenolic compound presence. However, phenolic compounds, including condensed tannins, represent only a 46% of the total DOC of leachates and further analyses need to be made in order to know whether leachate effects were caused by phenolic compounds or by other C-based compounds (see Chapter 6).

Changes in N availability under C. albidus

Besides the effects of plant presence and leaf leachates on changing N cycling processes we were also interested in studying potential negative effects of *C. albidus* presence on other plant species. Phenolic compound bearing species, including *Cistus* genera, may difficult the establishment of other plant species (Chaves and Escudero, 1997; Inderjit and Mallik, 1996b; Zackrisson and Nilsson, 1992). Moreover, *C. albidus* seems to exclude other species and its own germination since no vegetation is normally found under plants. Although several studies have been shown that phenolic compounds can decrease seed germination, root elongation or seedling establishment in experimental conditions (Chaves and Escudero, 1997; Inderjit and Mallik, 1996b; Nilsson and Zackrison, 1992), it is still not clear whether negative interactions among plant species mediated by secondary metabolites in natural conditions are caused by direct allelopathic effects to the target species or otherwise are due to indirect effects through changes in N availability (Michelsen *et al.*, 1995; Nilsson, 1994; Schmidt *et al.*, 1997; Wardle and Nilsson, 1997). We wanted to know whether *C. albidus* was decreasing N availability and thus outcompeting other plants with higher N requirements. We estimated N availability by net N mineralization rates on a dry mass basis. This seems an appropriate measure when comparing the same soils since no significant changes in bulk density are found. *C. albidus* presence increased N availability compared to control soil since individual plants concentrated organic matter in the soils beneath the canopy. Thus, the lower net N mineralization per unit of organic matter under *C. albidus* was counterbalanced by the higher organic matter released from plants. These "islands of fertility" induced by plant presence have been often observed in arid and semiarid areas (Vinton and Burke, 1995) while in other ecosystems plant presence have shown a decrease in N availability (boreal ecosystem- Chapter 4). The potential negative effects of *C. albidus* on other plant species through allelopathic interactions on target species needs further research.

The few studies available comparing phenolic compound production in plants growing on a acidity gradient in natural conditions have found higher phenolic concentrations in plants associated to lower pH (Gylphis and Puttick, 1989; Muller *et al.*, 1987; Nicolai, 1988; Northup *et al.*, 1995). There is no evidence that soil pH can have direct effects on phenolic compound synthesis, and this correlation is generally associated by changes in N availability, with lower N availability in acidic soils (Northup *et al.*, 1995). Results from our study follow the same trend as previous studies related to soil pH, with higher phenolic compound concentrations in *C. albidus* growing on siliceous soils compared to individuals growing on calcareous soils. However, foliar phenolic compounds were positively correlated with soil N availability and thus leaf N content. Moreover, phenolic compound concentrations within soil decreased in summer when plants were subjected to lower N availability.

Changes in carbon-based secondary compounds (CBSC), such as phenolic compounds, related to nutrient availability have been explained by resource allocation hypotheses: Carbon-Nutrient Balance (Bryant *et al.*, 1983) and Growth-Differentiation Balance (Herms and Mattson, 1992). These hypotheses predict increases in phenolic compound concentrations when N availability decreases or C availability increases. In those cases growth is more restricted than photosynthesis and the C excess is allocated to the synthesis of total non-structural carbohydrates (TNC), which are precursors of CBSC. Empirical data on the effects of N fertilization on CBSC generally support the prediction of Carbon-Nutrient balance (Jones and Hartley, 1999; Koricheva *et al.*, 1998) but have also failed in other studies (see review in Peñuelas and Estiarte, 1998). The amount of N availability is expected to determine the response of CBSC production. At low resources availability photosynthesis is highly constrained, and essential primary metabolic processes and baseline maintenance may receive priority in the use of limited C. In these conditions, rates of net assimilation, growth and secondary metabolism are positively correlated (Herms and Mattson, 1992). This could be the case of *C. albidus*, where differences in N-limitation among siliceous and calcareous soils could determine a parallel increase of phenolic compound concentrations and leaf N concentrations.

There are other factors besides soil nutrient status that could explain increases in phenolic compound concentrations, such as water stress (Gershenzon, 1984; Tang *et al.*, 1995). However, analyses of foliar $\delta^{13}C$, which is a good estimator of water use efficiency (Farquhar *et al.*, 1989), showed no differences in water availability among soils. Phenolic

compound concentration changes among and within soils, thus, are likely to depend on soil N status, with positive relationships between both factors.

Is there a positive feedback between soil N availability and plant phenolic compounds?

Since phenolic compounds and soil N availability can affect themselves reciprocally, some authors have hypothesized that high plant phenolic compound concentrations could be selected during evolution in those ecosystems with low N availability (Bending and Read, 1996; Northup *et al.*, 1995, 1998). These authors suggest that high phenolic compound concentrations in plants growing on extremely acidic, infertile soils could benefit productivity in those soils by sequestering N into a large unavailable pool of recalcitrant organic matter and thus minimizing N losses. Since phenolic compounds can decrease N availability by delaying the N cycling when they bound proteins and other N organic compounds (Bending and Read, 1996; Oades, 1988), they would have a conservative function of N in the ecosystem (Horner *et al.*, 1988).

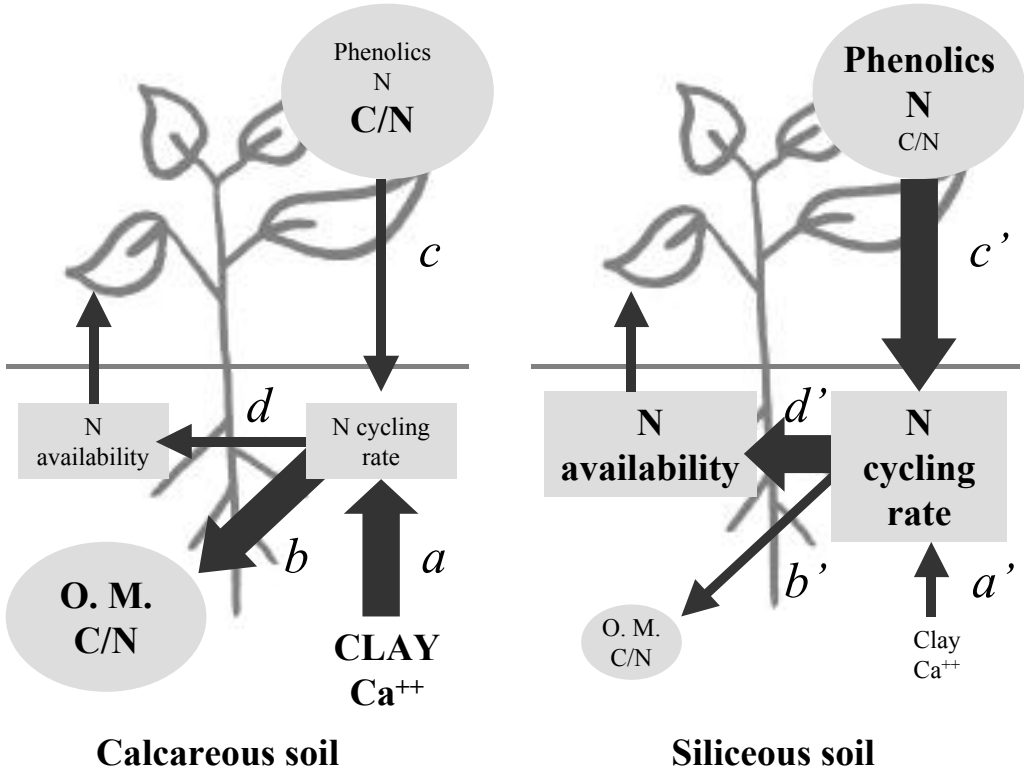

Figure 26. Plant-soil interactions for *C. albidus* growing on calcareous and siliceous soils (granitic and schistic). Arrows width and letter size represent the proportional strength of the described process. For more details see the text.

Our data suggest that a positive feedback between phenolic compounds and N conservation in the soil system is not likely between *C. albidus* and soils in the studied Mediterranean ecosystem. First, although a negative relationship between phenolic compounds and N availability is expected from the "positive-feedback hypothesis", *C. albidus* growing on a N-poor calcareous soil had lower phenolic compound concentrations than plants growing on the N-rich acidic soil (Fig. 26). Second, this hypothesis is based upon the capacity of phenolic compounds in binding organic matter and thus decreasing gross N mineralization. However, decreases in net N mineralization associated with *C. albidus* presence were probably due to increases in N immobilization since higher C mineralization and lower N-to-C mineralization was found in soils sampled under plants. Although the effect of phenolic compounds by binding organic matter and decreasing the pool and form of nutrients has been proposed to be the major link between phenolic compounds and nutrient cycling (Hättenschwiler and Vitousek, 2000), some studies found greater effects on increasing microbial activity when biota used phenolic compounds as a C source (Blum, 1998; Schmidt *et al.*, 1997; Sparling *et al.*, 1981; Sugai and Schimel, 1993).

Third, conservation of nutrients in the studied soil system were related to soil physical and chemical properties independently of phenolic compound presence. In calcareous soil, the slower microbial turnover due to high clay content was the main factor enhancing organic matter retention and thus conservation of nutrients in the soil, while in siliceous soils, which had low clay content, this process was not so relevant. Plant-soil interactions between *C. albidus* and siliceous and calcareous soils are described in Figure 8. In calcareous soils, the high content of clays and polyvalent cations stabilized organic matter and slowed N cycling rates (*a*), resulting in an accumulation of organic matter (*b*). Litter inputs from the plant represented a low proportion of the total organic matter present in the soil. Thus, plant effects (*c*) were weaker than physical and chemical effects in the calcareous soils (*a*). The slow N cycling produced a low N availability (*d*), which in turn influenced leaf chemical composition: plants had lower leaf N content and higher C/N ratio, and phenolic compound synthesis was reduced. In siliceous soils (granite and schist), the low clays and polyvalent cations content had a weak effect on N cycling rates (*a'*), so that N cycling rates were higher than in calcareous soils and the organic matter retention was lower (*b'*). Inputs of organic matter from the plant were expected to be similar compared to calcareous soils since no differences in plant biomass per soil area was found, but they were proportionally higher than soil organic matter content. Thus, in siliceous soils, the lower effect of soil physical and chemical properties on N cycling rate (*a'*) determined a higher proportional effect of plant presence (*c'*)

than in calcareous soils. Decomposition and mineralization was faster in siliceous soils and the resulting higher N availability (*d'*) caused higher leaf N and lower C/N ratio than in plants growing on calcareous soils. Within siliceous soils, the plant effect was stronger in granite compared to schist since the latter had higher clay content.

In summary, in the studied Mediterranean ecosystem it is not likely that higher plant phenolic compound concentrations had been selected during evolution in order to increase N retention in nutrient limited soils because (i) leaf phenolic compound concentrations were not negatively correlated with soil N availability, (ii) the strongest effect on N cycling of carbon compounds released from *C. albidus*, which include high phenolic compound concentrations, was an increased N immobilization but not a decreased in gross N mineralization, and (iii) the retention of organic matter in soil was more determined by soil physical and chemical properties than by plant phenolic compounds. Although we found that the release of C compounds from plants affected strongly N cycling, plant-soil interactions seem not to be the main evolutionary drivers for phenolic compounds presence and concentrations in the studied species. We conclude that the role of phenolic compounds in the ecological interactions of plants and their biotic environment, including the improvement of soil nutrient availability, antiherbivore defense, or allelopathy, will depend on the studied species and the ecosystem where this species evolved.

Acknowledgments

The authors would like to thank Marta Mastrantonio, Jordi Martínez, Roger Puig, Silvia Artesero, Bernat Castells and Francesc Cebrià for field assistance. We also thank David W. Valentine and Josep Maria Alcañiz for critically reading this manuscript, and Oriol Ortiz for his expertise help in soil analyses. E.C. has a predoctoral fellowship (FPI) from the Ministerio de Educacion y Cultura (Spain) in collaboration with Carburos Metalicos SA. We thank financial support from CICYT grants CLI99-0479 and REN-2000-0278/CLI (Spanish Government), IMMPACTE grant (DURSI and DMA, Catalan Government) and Fundació Territori i Paisatge (Catalonia).

Annex

			$N\text{-}NH_4^+$		$N\text{-}NO_3^-$		Total inorganic N	
			Initial	Final	Initial	Final	Initial	Final
SOIL TYPE	SITE (PLANT PRESENCE)	LEACHATE						
Winter sampling								
GRANITE	Control	-	0.84 ± 0.21	0.12 ± 0.05	0.32 ± 0.20	22.20 ± 4.75	1.17 ± 0.41	22.32 ± 4.79
		+		0.46 ± 0.45		18.88 ± 5.94		19.35 ± 5.76
	Under *C. albidus*	-	2.64 ± 0.85	0.47 ± 0.02	0.50 ± 0.25	29.44 ± 6.34	3.13 ± 0.75	29.91 ± 6.33
		+		1.35 ± 0.81		26.89 ± 6.82		28.24 ± 6.68
SCHIST	Control	-	1.62 ± 0.73	0.19 ± 0.10	3.46 ± 0.79	65.20 ± 11.28	5.08 ± 1.42	65.40 ± 11.38
		+		0.22 ± 0.02		51.10 ± 10.85		51.32 ± 10.84
	Under *C. albidus*	-	1.92 ± 0.86	0.34 ± 0.17	0.47 ± 0.05	48.43 ± 2.10	2.40 ± 0.82	48.77 ± 2.14
		+		0.59 ± 0.20		35.00 ± 2.29		35.59 ± 2.12
CALCAREOUS	Control	-	1.14 ± 0.10	1.18 ± 0.11	0.13 ± 0.10	32.39 ± 4.72	1.27 ± 0.18	33.57 ± 4.83
		+		1.78 ± 0.15		26.77 ± 4.05		28.55 ± 4.20
	Under *C. albidus*	-	1.64 ± 0.03	2.61 ± 0.83	0.10 ± 0.05	36.19 ± 11.78	1.74 ± 0.03	38.80 ± 12.23
		+		2.77 ± 0.24		28.99 ± 11.46		31.76 ± 11.35

(continue)

			$N\text{-}NH_4^+$		$N\text{-}NO_3^-$		Total inorganic N	
			Initial	Final	Initial	Final	Initial	Final
SOIL TYPE	SITE (PLANT PRESENCE)	LEACHATE						
Summer sampling								
GRANITE	Control	-	0.56 ± 0.14	0.21 ± 0.07	0.70 ± 0.19	18.18 ± 2.17	1.26 ± 0.13	18.39 ± 2.22
		+		0.10 ± 0.07		16.42 ± 2.32		16.53 ± 2.34
	Under *C. albidus*	-	0.59 ± 0.11	0.52 ± 0.12	0.61 ± 0.08	19.15 ± 1.63	1.20 ± 0.15	19.67 ± 1.61
		+		0.19 ± 0.04		18.90 ± 1.87		19.09 ± 1.88
SCHIST	Control	-	1.74 ± 0.80	0.40 ± 0.13	1.52 ± 0.28	33.56 ± 3.94	3.26 ± 0.81	33.96 ± 3.95
		+		0.26 ± 0.10		31.53 ± 4.18		31.79 ± 4.25
	Under *C. albidus*	-	0.94 ± 0.23	0.58 ± 0.20	2.37 ± 0.45	34.65 ± 2.34	3.31 ± 0.50	35.23 ± 2.50
		+		0.63 ± 0.23		31.71 ± 2.65		32.34 ± 2.45
CALCAREOUS	Control	-	2.43 ± 0.18	0.74 ± 0.20	1.18 ± 0.91	16.90 ± 1.62	3.61 ± 1.00	17.64 ± 1.57
		+		0.94 ± 0.43		14.96 ± 1.20		15.89 ± 1.15
	Under *C. albidus*	-	1.32 ± 0.30	1.04 ± 0.37	0.8 ± 0.67	18.29 ± 3.79	2.12 ± 0.39	19.33 ± 3.48
		+		1.18 ± 0.32		15.08 ± 3.86		16.26 ± 3.65

Table II. Ammonium and nitrate concentrations (μg N g^{-1}DM) before and after a 28 days incubation at 25 ºC, of winter and summer soils sampled under *C. albidus* and in a nearby control site at granite, schist and calcareous substrates, and amended with distilled water (-) or leachate (+).