Nitrogen and carbon concentrations, and stable isotope ratios in Mediterranean shrubs growing in the proximity of a CO₂ spring

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Abstract

Seasonal changes in foliage nitrogen (N) and carbon (C) concentrations and δ¹⁵N and δ¹³C ratios were monitored during a year in Erica arborea, Myrtus communis and Juniperus communis co-occurring at a natural CO₂ spring (elevated [CO₂], about 700 μmol mol⁻¹) and at a nearby control site (ambient [CO₂], 360 μmol mol⁻¹) in a Mediterranean environment. Leaf N concentration was lower in elevated [CO₂] than in ambient [CO₂] for M. communis, higher for J. communis, and dependent on the season for E. arborea. Leaf C concentration was negatively affected by atmospheric CO₂ enrichment, regardless of the species. C/N ratio varied concomitantly to N. Leaves in elevated [CO₂] showed lower δ¹³C, and therefore likely lower water use efficiencies than leaves at the control site, regardless of the species, suggesting substantial photosynthetic acclimation under long-term CO₂-enriched atmosphere. Leaves of E. arborea showed lower values of δ¹⁵N under elevated [CO₂], but this was not the case of M. communis and J. communis foliage. The use of the resources and leaf chemical composition are affected by elevated [CO₂], but such an effect varies during the year, and is species-dependent. The seasonal dependency and species specificity suggest that plants are able to exploit different available water and N resources within Mediterranean sites.

Additional key words: elevated CO₂, Erica arborea, global change, Juniperus communis, Mediterranean ecosystems, Myrtus communis, water stress.

Introduction

The present increase of atmospheric CO₂ concentration ([CO₂]) is predicted to continue throughout the century due to human activities. Plant communities of Mediterranean-type ecosystems are expected to be particularly sensitive to this increase because they will also have to face more severe drought conditions due to the predicted greenhouse gas driven increase in mean global temperature at the surface of the earth and to a concurrent decrease in precipitation at Mediterranean latitudes forecast by General Circulation Models (e.g. Kattenburg et al. 1996).

Increased [CO₂] has been observed to affect chemical composition of plant material (e.g. Lincoln 1993). In particular, the within plant allocation of carbon (C) and nitrogen (N) may be modified by the greater C availability (Ceulemans and Mousseau 1994), inducing increased C/N ratio (McGuire et al. 1995, Peñuelas et al. 1997) and C-based secondary compounds of foliage (Peñuelas and Estiarte 1997), thus possibly affecting plant susceptibility to herbivores (Lincoln 1993) and litter decomposition rate (Cotrufo et al. 1999). However, the latter effect on decomposition is not clear because it seems that the amount of N retained in naturally senesced litter produced under elevated [CO₂] may vary too little to affect subsequent decomposition (Norby and Cotrufo 1998). In semi-arid environments, such as Mediterranean-type ecosystems, the forecast drier conditions for the next decades would presumably bring about an additional effect on nutrient cycling (Cotrufo et al. 1999). Possible changes in the N source and the relative activity of
different N cycle processes (losses and supplies) in the surrounding system, as a response to elevated [CO$_2$], could be assessed by plant $\delta^{15}$N value (Peñuelas and Estiarte 1997, 1998).

Partial stomatal closure is a common, although not universal, response to elevated [CO$_2$] (Eamus 1991). This decreased stomatal conductance allows plants to use water more efficiently by enhancing the ratio of leaf net photosynthetic rate to evapotranspiration, termed instantaneous water-use efficiency. This increased water-use efficiency, for which leaf $\delta^{13}$C is a widely used substitution in C$_3$ species (Farquhar et al. 1989), could be especially advantageous to Mediterranean plant growth and survival when water supply is limiting.

Uncertainties on the effects of elevated [CO$_2$] on foliage chemical composition and water-use efficiency arise because predictions have mainly been extrapolated from studies of short-term exposure of plants in manipulated environments (greenhouses and different kinds of chambers), but physiological processes might adjust or acclimate to long-term increase in [CO$_2$] (Saxe et al. 1998). Natural CO$_2$ springs are an option to expensive FACE (free-air CO$_2$ enrichment) experiments and offer an opportunity for studying long-term responses of entire plant communities to enriched [CO$_2$], being unique environments of intrinsic ecological interest (Hättenschwiler et al. 1997, Körner and Miglietta 1994, Miglietta et al. 1993). Quercus species at the CO$_2$ spring of Bossoleto (Italy) have shown altered C physiology under elevated [CO$_2$], the response being species specific. Higher isoprene emissions, concentrations of phenolic compounds and total non-structural carbohydrates, and C exchange rates have been found in plants (Quercus species) grown at elevated [CO$_2$] (Johnson et al. 1997, Tognetti et al. 1998, Stylinski and Oechel, personal communication). However, much less information is available for the shrub layer (Jones et al. 1995, Barták et al. 1999) which constitutes the great bulk of the vegetation in Mediterranean-type ecosystems (Di Castri 1981).

*Erica arborea*, *Myrtus communis* and *Juniperus communis* are widely distributed in Mediterranean-type ecosystems, and form together with other shrubs species the dominant biomass in large areas of macchia vegetation. It is therefore highly relevant to investigate the effects of environmental changes which may alter the C and N interactions in these Mediterranean shrubs and which might in turn influence ecological interactions with other organisms and the functioning of macchia-dominated ecosystems. This study investigates the effect of prolonged elevation of [CO$_2$] on C and N concentrations in leaves of the three species. We also determined whether variations in N acquisition, water use efficiency, and acclimation, assessed by leaf N and C stable isotope composition, of individuals grown in the proximity of a natural CO$_2$ spring differ from those of comparable shrubs of the same species grown nearby but exposed to ambient [CO$_2$]. We tested 1) whether changes in C and N concentrations, and isotopic signatures occur at elevated [CO$_2$] or there is a long-term acclimation over a period of generations, and 2) whether they are general or particular for different shrub species. We hypothesized that these changes can be related to site histories (soil N dynamics and plant development as influenced by elevated [CO$_2$]), reflecting differences in atmospheric [CO$_2$] between the CO$_2$ spring and the control site.

**Materials and methods**

Three common and widespread Mediterranean macchia shrub species, *Erica arborea* L., *Myrtus communis* L. and *Juniperus communis* L., growing at a natural CO$_2$ spring near Lajatico (Pisa, Italy) called "Il Borboi" (43°26’N, 10°42’E) were measured. The enriched area extends over 7,000 m$^2$. The area is covered by a coppiced stand dominated by *Quercus ilex* L., but in which *Quercus pubescens* Willd., and *Arbutus unedo* L. (and scattered individuals of *Quercus cerris* L. and *Fraxinus ornus* L.) and the shrub level (including *Smiulax aspera* L., *Cytisus scoparius* L., *Cistus salvifolius* L., *Genista sp.*, *Ligustrum vulgare* L., *Pistacia lentiscus* L. and *Phyllirea latifolia* L.) constitute the great bulk of the flora. 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 a. s. l. (see Raiesi Gahrooei et al. 1999, Tognetti et al. 2000a). Almost pure CO$_2$ emissions occur from a series of vents located along a narrow seasonal creek, where [CO$_2$] tends to decrease upslope. The vents emit small amount of H$_2$S, never exceeding 0.04 μmol mol$^{-1}$. The whole canopy of the studied plants around the CO$_2$ spring is 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 (Hättenschwiler et al. 1997).

The shrubs growing in close association in the proximity of the CO$_2$ spring were measured. Additional measurements were made at a control site chosen along the same creek about 150 m upstream. Thus roots of selected plants experienced similar soil environment and branches similar aerial environment, except for [CO$_2$]; see Tognetti et al. (2000a) for a [CO$_2$] map of the area. The area has non-calcareous, brown loamy clayey soils, developed from calcareous marl (pH 6 - 7) (Raiesi Gahrooei 1998). Total soil N and C/N ratio in the forest floor and mineral horizon (0 - 10 cm) are comparable at
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both sites (Raiæsi Gahrooe et al. 1999). The climate is
typical Mediterranean, with cool winters, relatively wet
fall and spring, and hot, dry summers; details on
environmental conditions during the experiment can be
found in Tognetti et al. (2000b). At both sites, shrubs of
similar exposure were selected. Measurements were made
on sunny leaves in the upper or outer canopy (individuals
were not shaded by dominant species). Individuals within
a species were of similar size.

Every two months during one year, shoots were collected
from six individuals of each species (E. arborea, M. communis and J. communis) at each site
(CO₂ spring and control), early in the morning. Mean leaf
longevity of the three shrubs is 2 - 3 years, bud break and
leaf abscission took place in April - May. To avoid
possible isotope differences due to phenological effects,
we sampled plants and leaves in the same stage of
development at both sites. The shoots were transported to
the laboratory where they were oven-dried at 60 °C to
constant mass (d.m.). Leaves were then ground to a fine
powder in a mill. Carbon and N leaf concentrations were
analysed with an elemental analyser (NA 1500, Carlo
Erba, Milano, Italy), using the standard configuration for
those determinations (Péruelas et al. 1999); 10 - 12
current-year leaves of the same stage of development
were bulked for each plant at each sampling date.
The δ¹⁵N and δ¹³C ratios were measured on sub-
samples (about 1 mg) of ground leaves by a Delta C
isotope ratio mass spectrometer (Finnigan Mat, Bremen,
Germany) operated in continuous flow mode, after
combustion of the samples in an elemental analyser (NA
1108, Series 1, Carlo Erba). A system check of analysis
was achieved with interspersed working standards of
acetanilide and atropine (Carlo Erba) for elemental
analyses, and standards of sucrose, graphite, polyethylene
fossil, ammonium sulphate and potassium nitrate (IAE,
Vienna, Austria) for isotopic analyses (Péruelas et al.
1999). The δ¹³C value for source atmospheric CO₂ during
the study averaged -8.3 % and -8.9 % at the control and
CO₂ spring site, respectively (M. Sauer, personal
communication); these average values are very similar
and relate to the same area and time over which the
average [CO₂] was assessed, also resembling data from
long-term geological surveying. The accuracy of the
measurement was ± 0.2 % for δ¹⁵N and ± 0.1 % for δ¹³C.

Within each species, two-way analysis of variance
(ANOVA) with sampling date and site (with different
[CO₂]) as the main effects were conducted for all cases
after testing for normality and homogeneity of variances.
A repeated-measure analysis was not considered
appropriate because we did not sample the same plants
over the sampling time period. Differences amongst the
three species were also tested with ANOVA. Statistical
analysis was conducted by using Statistica 5.5.

Results

In M. communis the N concentration was significantly
lower in leaves of CO₂ spring plants (Table 1), while in
J. communis it was lower (P < 0.05) in leaves of control
plants. In E. arborea it was consistently lower in leaves
of CO₂ spring plants in mid-winter only. Leaf N
concentration did not display consistent seasonal variation
throughout the year (Fig. 1). In general CO₂ spring plants
showed less seasonal variations than control plants.
Differences amongst species were significant (P < 0.001),
with J. communis (control plants) showing generally
lower values (N %) than E. arborea and M. communis.

Markedly lower C concentrations were generally
found in leaves of CO₂ spring plants (Table 1), regardless
of the species, but the leaf C concentration seasonal
course was not clear (Fig. 2). Differences amongst species
were strong (P < 0.0001), with E. arborea showing
constantly higher values (C %) than the other two species.

In M. communis the C/N ratio was overall
significantly higher in leaves of CO₂ spring plants
(Table 1), while in J. communis it was particularly higher
in leaves of control plants during late winter and early
spring (Fig. 3); leaves of E. arborea showed higher C/N
ratio in CO₂ spring than control plants only in late winter.
M. communis displayed increasing values of C/N ratio

from the fall to the next growing season. Again, CO₂
spring plants had overall less seasonal variations than
control plants. Differences in C/N ratio amongst species
were highly significant (P < 0.0001).

Table 1. Summary of analysis of variance (ANOVA) results showing the effects of [CO₂] (site), measuring month (month) and interactions amongst effects (s x m) on leaf chemical composition of three Mediterranean shrubs; differences between species are reported in the text. * P ≤ 0.05. ** P ≤ 0.01. *** P ≤ 0.001, **** P ≤ 0.0001, n.s. P > 0.05 (not significant).

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Fig. 1. Seasonal course of N concentration (% dry mass) measured in leaves of *Erica arborea*, *Myrtus communis* and *Juniperus communis* plants at the CO₂ spring site and at the control site during 1996 - 1997. *Vertical bars* indicate SE of the mean (*n* = 6 individuals). Species and sites are referred to by symbols in the legend.

Fig. 2. Seasonal course of C concentration (% dry mass) measured in leaves of *Erica arborea*, *Myrtus communis* and *Juniperus communis* plants at the CO₂ spring site and at the control site during 1996 - 1997. *Vertical bars* indicate SE (*n* = 6). Species and sites are referred to by symbols in the legend.

Fig. 3. Seasonal course of C/N ratio measured in leaves of *Erica arborea*, *Myrtus communis* and *Juniperus communis* plants at the CO₂ spring site and at the control site during 1996 - 1997. *Vertical bars* indicate SE (*n* = 6). Species and sites are referred to by symbols in the legend.

Fig. 4. Seasonal course of δ²⁴N measured in leaves of *Erica arborea*, *Myrtus communis* and *Juniperus communis* plants at the CO₂ spring site and at the control site during 1996 - 1997. *Vertical bars* indicate SE (*n* = 6). Species and sites are referred to by symbols in the legend.
Strong site differences in leaf δ¹⁵N values (%) were evident only in E. arborea (Table 1), with control plants showing generally higher values than CO₂ spring plants (except for late summer and early fall). Patterns of leaf δ¹⁵N in E. arborea showed a consistent minimum in late summer (Fig. 4), while the other two shrubs did not display significant seasonal changes (except for a maximum in early summer in M. communis CO₂ spring plants). Differences in δ¹⁵N values amongst species were highly significant (P < 0.0001).

The C stable isotope analyses yielded significantly lower values of δ¹³C in leaves of plants growing at the CO₂ spring site, regardless of the species (Table 1). Seasonal patterns were approximately constant for E. arborea (Fig. 5). Relatively larger variations were observed in M. communis and J. communis. There was significant variation (P < 0.0001) in δ¹³C amongst the leaves of E. arborea, M. communis and J. communis. J. communis had relatively higher δ¹³C than M. communis and E. arborea.

In E. arborea leaves, the higher δ¹⁵N values was somewhat accompanied by higher N concentrations (data not shown), though the relationships had low r² (0.16 and 0.25 for CO₂ spring and control plants, respectively); such relationships in the other two species showed even lower r², except for M. communis control plants (0.36). The plot of δ¹⁵N versus δ¹³C separated the two sites well (data not shown), regardless of the species. In general, at corresponding values of δ¹⁵N the leaves of CO₂ spring plants had more negative δ¹³C values. The range of δ¹⁵N values was larger in E. arborea than in the other two species. Higher average δ¹⁵N values were found in M. communis than in J. communis, while the opposite was the case for δ¹³C values.

Discussion

Even though total N concentration and C/N ratio do not differ between soil/litter at the CO₂ spring and at the control site (Raiesi Gahrooe et al. 1999), significant differences were found in leaf N concentrations. M. communis showed lower N concentration and higher C/N ratio in plants grown at the CO₂ spring site as described in most studies of elevated [CO₂] effects (McGuire et al. 1995, Péñuelas and Estiarte 1997, Péñuelas et al. 1997). However, this decrease in N concentration at elevated [CO₂] was not found in the other two studied species, specially in J. communis which showed increased N concentrations, so caution is required in the generalization of such a response among species.

While leaf C concentration was generally lower in CO₂ spring plants, the changes in the C/N ratio followed mainly the pattern of leaf N concentration, and therefore opposite trends between M. communis and J. communis and seasonal-dependent trends in E. arborea were observed. In all shrubs, carboxylation became more restricted relatively to diffusion through stomata, resulting in downward photosynthetic adjustment (Tognetti et al. 2000b). Plants of semi-arid environments may need to prioritise water saving over C gain. It must be pointed out that differences between sites in leaf N and C concentration (and C/N ratio), if any, may disappear depending on the period of sampling (Péñuelas et al. 1997) and, possibly, if allometric relations are considered. Indeed, elevated [CO₂] may cause a shift in the allocation pattern of carbon among leaves, woody stems, fine roots, and rhizomes.

The potential significance of CO₂-induced changes in leaf quality for herbivore feeding and development (Lincoln et al. 1993), for litter decomposition (Cotrufo et al. 1999) and for C sequestration in soils (Schimel 1995) has been highlighted. In M. communis the observed decrease in N concentration may result in herbivore stress under conditions of increased [CO₂] (Lincoln 1993). However, the results of this investigation suggest caution in linking changes in leaf nutrient concentrations and these phenomena under long-term [CO₂] exposure. The water stress experienced by these drought-adapted plants (Tognetti et al. 2000b) might have been insufficient to
produce an evident decline in the acquisition of resources or a change in the C/N ratio, in correspondence with the balance of chemical compounds. Variations in tissue nutrient concentration (and resource use) among plants grown at elevated or ambient [CO₂] may depend on the effect of [CO₂] on plant size (growth and development); other woody plants at the same location did not show differences in size between the CO₂ spring and the control site (Tognetti et al. 2000a).

All major pathways of ecosystem N loss (nitrification, denitrification, ammonia volatilization and nitrate leaching) are thought to cause a ¹⁵N enrichment of the remaining N and a ¹⁴N depletion of the lost N (Peñuelas et al. 1999). E. arborea had lower leaf δ¹⁵N in plants at the CO₂ spring site which may thus indicate higher activities of N-input processes and/or lower activities of N-losing processes in the soil system surrounding the roots, possibly because of higher plant demand under elevated [CO₂]. These processes, however, do not necessarily produce lower soil δ¹⁴N values and the effect of the processes will depend on the δ¹⁵N values of the inputs and of soil-N pool being affected. A preferential C partitioning to below-ground structures under elevated [CO₂] (greater root/shoot ratio) has been sometimes proposed (Ceulemans and Mousseau 1994, Saxe et al. 1998). The soil N fractionation activity of mycorrhizal fungi of this species may also be affected by elevated [CO₂]. Again, the response to elevated [CO₂] was different in the other two studied species; they showed no clear pattern (J. communis) or it was season-dependent (M. communis). The between species differences in leaf δ¹⁵N values reported here, although not strong, are probably a consequence of specific differences in the exploitation of N resources. M. communis, showing less negative averaged leaf δ¹⁴N than the other two species, also had field water relations indicating relatively deeper roots (Tognetti et al. 2000b) which would allow this species to use N from more N-saturated soil layers (Lloret et al. 1999).

There was not strong variation in C stable isotope composition amongst the leaves of E. arborea, M. communis and J. communis, which showed for plants at the control site values similar to those reported for other C₃ species of macchia vegetation (Valentini et al. 1992). The variation between sites seemed more important in determining leaf C stable isotope composition than stable isotope composition (δ¹³C values of the source CO₂ were similar between sites compared to the differences seen in plants, environmental factors being the same except for [CO₂], see Materials and methods). In the three species studied δ¹⁵N values for CO₂ spring plants were more negative than for plants at the control site, and therefore elevated [CO₂] has resulted in “consequent” lower δ¹⁵N-derived water use efficiency values. The depletion of ¹³C, as measured in the products of photosynthesis, is modulated by the rate at which CO₂ diffuses into the leaf and by the rate at which CO₂ is fixed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Farquhar et al. 1989). Provided that stomatal conductance of plants grown at the CO₂ spring site was lower than in control plants (Tognetti et al. 2000b), stable isotope analysis suggests photosynthetic acclimation under long-term CO₂-enriched atmosphere (Miglietta et al. 1998). Previous studies on gas exchange and growth of evergreens grown at CO₂ springs fit with δ¹³C data, as reported in this study, and support this hypothesis. The down-regulated photosynthetic rates might reduce the usually observed benefit of increased water use efficiency associated with growth under elevated [CO₂] (Eamus 1991). The Mediterranean sclerophylls studied in this experiment display an intrinsic growth strategy that highly prioritizes water saving over C uptake. Long-term water use efficiency, however, might be affected by different proportion of respired versus fixed C between CO₂ spring and control plants, when derived by C stable isotope analysis. Several studies have shown that contents of C-based secondary compounds increase in plants grown under elevated [CO₂] (e.g. Lavola et al. 1994), while others have found no significant response (e.g. Johnson and Lincoln 1990). Due to their biosynthetic pathway, C-based secondary metabolites are characterized by lower δ¹³C than carbohydrates. Indeed, changes in concentration of phenolic compounds and total non-structural sugars and emission of volatile organic compounds have been found for leaves of Quercus species grown in another CO₂ spring, though differences between species in magnitude and direction of such changes were observed (Tognetti et al. 1998). The plot of leaf δ¹⁵N versus leaf N concentration did not indicate clear changes in N availability with variations in N-loss activity, regardless of species and site. In plants, δ¹³C has been used as an index of stress tolerance (water loss versus carbon gain) and δ¹⁵N is genetically and environmentally determined (rather than being simply a function of the isotopic signature of external N source). The plot of leaf δ¹⁵N versus δ¹³C values separates the two sites well, regardless of the species, and the separation is due to differences in δ¹³C. The variation in the isotopic signature reveals different strategies with respect to water use (and N use in E. arborea) between plants at the CO₂ spring and control site. Environmental factors, which decrease the proportional flux of plant N into organic matter storage pools, may push the plant N toward δ¹⁵N-enrichment, relative to atmospheric N.

In conclusion, reduced N concentration and related changes in C/N ratio are not a general response to long-term exposure to elevated [CO₂] in these Mediterranean shrubs. Instead, acclimation to elevated [CO₂] of C metabolism, with relative lack of excess C, may result
References


