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Elevated CO_2 and nitrogen supply alter leaf longevity of grassland species

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Summary

• The longevity of green leaf area for 10 grassland species was measured to establish whether elevated CO_2 and N fertilization alter leaf longevity, an important determinant of ecosystem leaf area and ecosystem carbon gain.

• Plants were selected from monocultures in their second year of growth in a field experiment that directly manipulated atmospheric CO_2 (550 ppm and ambient) and nitrogen fertilization (4 g N m^{-2} and ambient). Leaves were censused biweekly over a 4-month period.

• Leaf longevity increased under elevated CO_2 (+3.4 d, $P = 0.03$) and decreased under elevated N (−4.2 d, $P = 0.03$). Leaf longevity increased under elevated CO_2 for C_3 species only; there was no change in leaf longevity of C_4 species under elevated CO_2. For both CO_2 and N, changes in leaf longevity were congruent with expectations based on observed changes in N cycling.

• In addition to supplies of resources such as CO_2 and N, site fertility and the development of ecosystem feedbacks appear to be important in determining leaf longevity.

Key words: Atmospheric CO_2, Cedar Creek Natural History Area, grasslands, leaf longevity, N fertilization.

Introduction

CO_2 and N supplies to ecosystems are increasing worldwide (Vitousek *et al.*, 1997; Koch & Mooney, 1996). Mechanistic understanding of the responses of ecosystems to changes in resource supplies requires mechanistic understanding of ecosystem C and N cycles. Although many plant traits affect C and N cycles in an ecosystem, leaf longevity has a central role in determining litterfall, standing biomass, and net primary productivity (NPP). For example, increases in leaf longevity could increase the carbon gain of individual leaves (assuming no other changes in factors that affect leaf carbon gain) and therefore increase stand NPP. Changes in CO_2 and N supplies to grasslands have the potential to alter leaf longevity and therefore key ecosystem properties, yet data on grassland leaf longevity and its responses to changes in resource supplies are scarce (Craine *et al.*, 1999; Knapp *et al.*, 1999).

In general, greater leaf CO_2 economy and shoot growth typically lead to shorter leaf life-span (Reich, 1998). At the leaf level, photosynthetic rate under light-saturated conditions, specific leaf area (SLA), and leaf tissue N concentration are negatively associated with leaf longevity among species (Reich *et al.*, 1997). Under most situations, leaf longevity is also negatively associated with nutrient supplies (Shaver, 1983), light availability (Reich, 1995; Reich *et al.*, 1995), and more favourable temperatures and related microenvironmental factors (Schoettle, 1990).

These data can be used to predict the effects of N and CO_2 in grasslands. As increased N supply should also lead to increases in photosynthesis and leaf N concentrations, we hypothesize that leaf longevity for grassland species decreases with increases in N supply. Elevated CO_2 can have many direct and indirect effects on plant and ecosystem parameters that could lead to either increases or decreases in leaf longevity. Elevated CO_2 typically increases photosynthetic rates and plant growth rates (Koch & Mooney, 1996; Curtis & Wang, 1998), which would presumably be associated with decreases in leaf longevity. Yet, elevated CO_2 generally decreases tissue N concentration and SLA (Curtis, 1996; Norby *et al.*, 1999; Roumet *et al.*, 1999), which are associated with greater leaf longevity. Elevated CO_2 also leads to decreased transpiration and higher soil moisture (Jackson *et al.*, 1998; Knapp, 1993)

and therefore more favourable plant water status (Knapp *et al.*, 1999) which could increase leaf longevity if drought has led to accelerated senescence. Alternatively, the increases in soil moisture and plant water status could decrease longevity if the primary effect is to increase photosynthetic rates. The few studies that have examined leaf longevity and senescence on grasses have generally shown no effect of elevated CO_2 on leaf longevity, or longevity was greater under elevated CO_2 (Curtis *et al.*, 1989; Knapp *et al.*, 1999; Navas *et al.*, 1999). Due to the multiple effects of elevated CO_2 on plant and ecosystem functioning, there is currently no clear prediction for the response of leaf longevity to elevated CO_2. For both N and CO_2, responses may depend on the functional characteristics of species (e.g. C_3 vs C_4 photosynthetic pathways) and there is a need to examine the responses of many species to increases in CO_2 and N supplies.

We measured the longevity of leaf area of 10 herbaceous species that were grown in two-year-old monocultures exposed to elevated CO_2 and N supplies in a factorial manner. We employed techniques modified from Craine *et al.* (1999) in order to determine the longevity of green leaf area, rather than entire leaves. This eliminates potential bias in longevity that is due to differences in life-span among leaves of different sizes for an individual and provides data that are more amenable to use in ecosystem models. We also compare the data on leaf area longevity for 7 of the 10 species with previously collected leaf longevity data from nearby older monocultures on a lower fertility site. For these two data sets, we examine associated leaf, whole-plant and ecosystem measures to better understand the nature of variation in among species and experiments.

Materials and Methods

BioCON design

We addressed issues of the response of leaf area longevity to changes in CO_2 and N supplies by measuring in a subset of monoculture plots for 10 species of a larger study of elevated CO_2, N fertilization, and decreases in biodiversity (the BioCON experiment, Reich *et al.*, 2001). Each plot is 2 m × 2 m, situated on low-N, sandy soils, and distributed among six 20-m diameter experimental areas (rings). Each monoculture is replicated twice at two factorial levels of N and CO_2 supplies. Monocultures were planted in 1997 with 12 g m^{-2} of seed. Throughout the 1998 growing season, three rings were exposed to ambient atmospheric CO_2 concentrations and three to elevated CO_2 using the free-air CO_2 enrichment system (Lewin *et al.*, 1994). In the three elevated CO_2 rings, sufficient CO_2 was added during daylight hours to maintain the atmosphere at 550 μmol CO_2 mol^{-1}; the three ambient rings were treated identically but without the additional CO_2. Half of the plots were amended with 4 g N m^{-2} yr^{-1} in the form of NH_4NO_3 applied over three dates. CO_2 treatment began in April, 1998 and the first N treatment began in May 1998.

Leaf longevity was measured in monocultures of 10 species: 4 C_3 grasses (*Agropyron repens*, *Bromus inermis*, *Koeleria cristata*, and *Poa pratensis*), 4 C_4 grasses (*Andropogon gerardi*, *Bouteloua gracilis*, *Schizachyrium scoparium*, and *Sorghastrum nutans*) and 2 C_3 forbs (*Achillea millefolium* and *Solidago rigida*) with similar census techniques as Craine *et al.* (1999). Leaf longevity was determined on three randomly chosen individual plants in each plot. A grass individual was defined as a set of leaves that appeared to share the same basal meristem; a forb individual was defined as those leaves that shared the same stem emerging from the ground. Plants were first censused on day 139 of 1998 (May 19) and recensused every 2 wk until day 258 of 1998 (September 15). This period represents the majority of the growing season for the 10 species we sampled. Leaves that had been damaged by herbivory or other factors were removed from the data set. Individual plants that had died were replaced by new plants. Less than 5% of the individuals were replaced and < 2% of all leaves were excluded from the analysis due to damage.

For forbs, for each census date, if a leaf was judged to be approx. fully expanded, the date was recorded as the 'birth' date, a numbered 1 × 2 cm paper tag was attached at the base of the leaf with floss, and the maximum width and length of the leaf recorded. We estimate that, on average, expansion of forb leaves required *c.* 2 wk. During the census, the maximum width and length of previously tagged leaves were recorded, previously marked leaves were classified as being green or senesced, and new leaves tagged and measured. All leaves that were present at the beginning of the experiment or subsequently produced on the selected individual during the census period were tagged and quantified in this manner.

New grass leaves were marked at the base of the leaf with a permanent marker to uniquely identify each leaf on an individual plant that had been demarcated with its own tag. There was no observed difference between the longevity of marked leaves in comparison to unmarked leaves on adjacent plants. With each census date, the average width of the leaf, the total length of the leaf and the length of the senesced portion of the leaf were recorded. The senesced length was determined as the leaf length from the leaf tip to the interface of the green non-senescent tissue and the brown, senescent tissue. If the senescence was noncontinuous, the total senescence length was measured in an according manner.

After leaves were measured, the corresponding areas of the leaves or sections of leaves were determined. For each grass leaf, the green and senesced areas were determined as the product of the width of the grass leaf and the length of the green or senesced portion of the leaf. For each forb species, we developed an allometric equation that predicts leaf area from length and maximum width. 12–19 leaves of various sizes from each of the four ambient CO_2 plots of each forb species were collected in September, 1999. Subsequently, the length, maximum width, and area were determined for each leaf. Leaf area was determined with a Li-Cor LI-3000 Leaf Area Meter

(Li-Cor Inc., Lincoln NE). For each species, a stepwise regression model was run for leaf area with length, width, and the squares of both measures. For *Solidago rigida*, the equation for predicting area was: $-0.22 + 0.075$ Length^2 + 1.3 Width^2 ($r^2 = 0.99$). For *A. millefolium*, the equation for predicting area was: 0.043 + 0.011 Length^2 + 0.60 Width^2 ($r^2 = 0.91$). N treatment was not found to affect the relationships between area and length or width.

The calculations that we used to determine the longevity of an average unit of leaf area were derived from Craine *et al.* (1999). The new calculations reflect using leaf area as a standard unit to compare species instead of individual leaves for forbs or leaf length for grasses and eliminate any bias that might be associated with an individual's leaves of different sizes having different life-spans. In brief, the total leaf area that was both born and senesced in a plot over the census period and data on the dates of the censuses are used to calculate the total number of days lived by all the leaf area (leaf area-days) both born and senesced during the period. Division of the leaf area-days of all the leaves in a plot by the area of the leaves that had been both born and senesced during the census period gives the average longevity of the leaf area that had been both born and senesced during the census period. This calculation of the longevity of leaf area represents an average longevity over the census period, but does not compare longevity at different times of the year that may be associated with seasonal or ontogenetic differences among species.

Using data on the total leaf area present at each census, determining leaf area longevity first required determining the total number of leaf area-days that had been both born and senesced during the census using the following equation:

$$\left(\sum_{n=i}^{j}\left(\frac{A_{n+1}+A_n}{2}\right)\times k_n\right)- \quad \text{Eqn 1a}$$

$$\left(\sum_{n=l}^{j}\left(\frac{S_{n+1}+S_n}{2}\right)\times k_n\right)- \quad \text{Eqn 1b}$$

$$\left(((n_s-n_l)\times L_l)-\left(\sum_{n=l}^{s}\frac{S_{n+1}+S_n}{2}\times k_n\right)\right)- \quad \text{Eqn 1c}$$

$$\left(((n_j-n_l)\times S_j)-\left(\sum_{n=l}^{j}\frac{A_{n+1}+A_n}{2}\times k_n\right)\right) \quad \text{Eqn 1d}$$

(i, first census date; j, last census date; A_n, cumulative leaf area observed or produced by census date n; A_{n+1}, cumulative leaf area observed or produced by the census following census n; S_n, cumulative leaf area senesced by census date n; S_{n+1}, cumulative leaf area senesced by the census following census n; k_n, time interval between census n and $n+1$; n_S, the date at which the amount of leaf area senesced is equivalent to the amount of leaf area at the first census ($S = A_i$); and n_l, the date at which the cumulative leaf area is equal to the amount of leaf area senesced by the last census date ($A = S_j$).)

The first two parts of the equation represent the total number of leaf area-days for all leaves tracked during the census, which is calculated as the difference between the total number of leaf area-days for total (Eqn 1a, Fig. 1a) and senesced leaves (Eqn 1b, Fig. 1b) and is equivalent to the area between the curves for the census period used. The second part of the equation (Eqn 1c, Fig. 1c) represents the number of leaf-days for leaves that may have been present at the beginning of the census period, but should not be included in

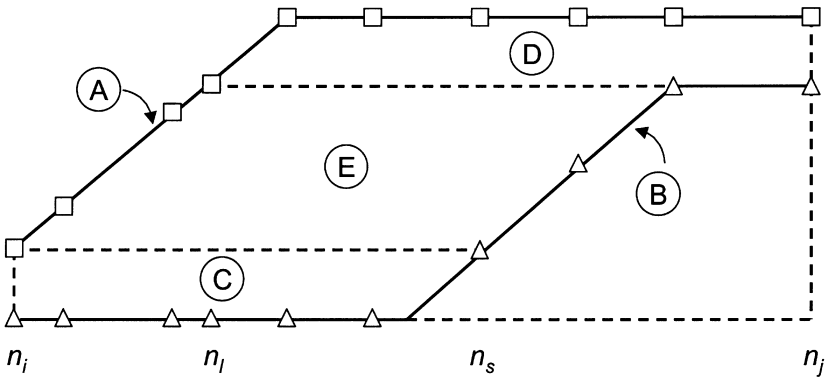

Fig. 1 Calculation of leaf area longevity of a species. Curve A represents the cumulative amount of leaf area (green or senesced) that was observed over the entire census period (Eqn 1a). Curve B represents the cumulative amount of senesced leaf area over the entire census period (Eqn 1b). The areas under the curves are the total number of days that leaf area was green or senesced (Area under A) or the total number of days that leaf area was senesced (Area under B). The difference between these two curves represents the total number of leaf area-days for unsenesced leaves. Area C represents the total number of leaf area-days for leaves that were present at the beginning of the census (date of birth unknown) (Eqn 1c). Area D represents the total number of leaf area days for leaves that did not senesce during the census period (date of death unknown) (Eqn 1d). Subtracting Areas C and D from the area between Curves A and B provides the total number of leaf area days for leaves that were both born and senesced within the census period (Area E). Dividing Area E by the total area of leaves in Area E (the height of E) provides the estimate of leaf area longevity, equivalent to the average horizontal distance of Area E. Also represented are the first (n_i) and last (n_j) census dates and the interpolated 'census dates' n_l and n_s (see text for details).

the determination of leaf area longevity as the date of birth of the leaf area was unknown and hence leaf area longevity could not be determined. The third part of the equation (Eqn 1d, Fig. 1d) represents the number of leaf area-days for leaves that had not senesced by the end of the census period. Similarly, this leaf area should not be included in the calculations of leaf area longevity as it is unknown when this leaf area would have senesced. These two quantities are subtracted from the total number of leaf area-days to provide the total number of leaf area-days for only those leaves that were both produced and senesced within the census period (Fig. 1e). Leaf area longevity is equivalent to the average horizontal distance between the two curves and was calculated by dividing the censused leaf area-days for which birth and death dates are known (Eqn 1a–d) by the total amount of leaf area that was born and senesced during the census ($A_i - A_j$). The dates n_l and n_s were calculated by linear interpolation based on the relevant census measures and dates. All analyses were computed with JMP vs 3.2.2 (SAS Institute).

For each species, average leaf area longevity and the standard errors of the means were computed with plots serving as replicates for a species. As an index of the total amount of leaf area that we sampled for a species, we also have provided the mean and standard error of leaf area sampled per individual that had both been produced and senesced during the census period. The average leaf area longevity and leaf area sampled were also calculated for all species at each level of CO_2 and each N level. Differences in leaf area longevity and leaf area among species and treatments were determined with a linear regression model. Leaf area longevity was modelled with an additive general regression model that included the functional group of the species, the identity of the species (nested within functional group), the CO_2 treatment category, the N treatment category, the interaction between CO_2 and N treatment categories, and the interactions between functional group identity and both CO_2 and N treatments. Due to the few number of replicates for each species at each factorial combination, we did not test for species-specific responses to elevated CO_2 or N (i.e. no CO_2 * species or N * species interactions).

In 5-yr-old monocultures (Cedar Creek LTER experiment E111), we had measured leaf longevity for 7 species that were also measured in the BioCON study (*A. repens*, *A. gerardi*, *K. cristata*, *P. pratensis*, *S. scoparium*, *S. rigida*, and *S. nutans*) using equivalent techniques (Craine *et al.*, 1999). Soils in the 5-yr-old monocultures had similar amounts of soil N 0–20 cm (0.063%), but less soil C 0–20 cm (0.47 vs, 0.57% for BioCON) (Wedin, unpublished; Reich, unpublished). Even after 5 yr, biomass was much less in E111 than BioCON (Table 2), indicating much lower productivity in E111 than BioCON. We also compare average leaf percentage N (Reich, unpublished), root biomass, mid-June extractable NO_3^-, and N mineralization rate mid-June to mid-July (Reich *et al.*, 2001) for the 7 species from BioCON and the 5-yr-old monocultures (Craine, unpublished) with *t*-tests and correlations. To examine the relationship of leaf longevity and these 4 measures among the 7 species in BioCON we ran a principal components analysis with the BioCON data and examined the loadings of these measures on the first axis.

Results

On average *c.* 20 cm^2 of leaf area was sampled per individual (Table 1). In general, variation in leaf area for a species was related to the size of individual leaves, not the total number of leaves sampled (data not shown). Fertilization increased leaf area production by 30%, while elevated CO_2 did not lead to any change in leaf area production (Table 2).

The average leaf area longevity for species ranged from 36.1 d (*A. millefolium*) to 63.9 d (*S. rigida*) (Table 1). Among functional groups, the average leaf area longevity of forbs was 50.0 d, C_3 grasses 55.6 d, and C_4 grasses 42.4 d (Table 1). The statistical model of leaf area longevity explained 68% of the total variation in leaf area longevity among samples. Together, functional group identity and the species identity explained most of the variation (Table 2) with each explaining a similar

Table 1 Mean (± SE) longevity of a unit of leaf area and leaf area production per individual over the measurement period and also means across functional groups

	n	Longevity (d)	Area (cm^2)
Forb		50.0 ± 2.5	22.0 ± 2.5
Achillea millefolium	8	36.1 ± 5.5	10.2 ± 1.1
Solidago rigida	8	63.9 ± 4.4	33.8 ± 6.8
C_3 grass		55.6 ± 1.8	15.1 ± 1.8
Agropyron repens	8	57.5 ± 3.6	24.1 ± 4.2
Bromus inermis	8	59.8 ± 2.2	24.4 ± 4.4
Koeleria cristata	8	42.5 ± 3.4	6.3 ± 1.4
Poa pratensis	8	62.6 ± 3.2	5.8 ± 1.0
C_4 grass		42.4 ± 1.8	22.5 ± 1.9
Andropogon gerardi	8	37.1 ± 2.8	37.2 ± 4.8
Bouteloua gracilis	7	31.0 ± 1.4	6.2 ± 0.7
Schizachyrium scoparium	8	43.3 ± 4.9	12.8 ± 1.6
Sorghastrum nutans	7	58.3 ± 2.7	33.2 ± 4.5
CO_2			
Ambient CO_2	40	47.5 ± 2.3	20.5 ± 2.6
Elevated CO_2	38	51.3 ± 2.5	18.2 ± 2.4
Nitrogen			
Ambient N	40	51.3 ± 2.5	16.9 ± 2.4
Elevated N	38	47.2 ± 2.4	22.0 ± 2.5
CO_2 * Functional			
C_3 grass (ambient)	16	53.4 ± 2.4	16.0 ± 2.5
C_3 grass (elevated)	16	57.3 ± 2.4	14.3 ± 2.5
C_4 grass (ambient)	16	43.3 ± 2.4	23.0 ± 2.5
C_4 grass (elevated)	14	41.3 ± 2.6	21.5 ± 2.7
C_3 forb (ambient)	8	42.9 ± 3.4	24.7 ± 3.5
C_3 forb (elevated)	8	57.1 ± 3.4	19.3 ± 3.5

Least squares means are included for the functional groups at the different CO_2 levels, but not for N treatment as the interaction between functional group and N treatment was not significant for either leaf longevity or leaf area production.

Table 2 Results of the model that predicts leaf longevity

	Longevity		Area	
	F ratio	Prob > F	F ratio	Prob > F
Fxnl	14.5	< 0.001	4.6	0.01
Species[Fxnl]	12.1	< 0.001	14.3	< 0.001
CO_2	5.1	0.03	1.4	0.23
Nitrogen	4.8	0.03	5.0	0.03
CO_2 * Nitrogen	0.0	0.99	0.1	0.79
CO_2 * Fxnl	3.7	0.03	0.2	0.79
N * Fxnl	0.9	0.40	2.3	0.11

Predictor variables include functional group (Fxnl), species identity nested within functional group, CO_2 treatment, N treatment, and the interaction between CO_2 and N. For leaf longevity, $r^2 = 0.68$. For leaf area, $r^2 = 0.67$.

amount of variation. Leaf area longevity increased under elevated CO_2 (+3.4 d, $P = 0.03$) and decreased under elevated N (−4.2 d, $P = 0.03$). The interaction between functional group identity and CO_2 was significant (Table 2) with C_3 forbs showing the greatest increase in leaf area longevity, C_3 grasses a moderate increase in leaf area longevity and no increase in leaf area longevity for C_4 grasses.

Analysis of data from the older monocultures grown on the lower fertility soil of E111 reveals the need to understand better the role of site fertility and species traits in determining not only leaf longevity but other plant and ecosystem properties. For ease of discussion, when comparing the two experiments we'll refer to both leaf area longevity of BioCON and the leaf longevity of E111 as leaf longevity. Leaf longevity was lower in BioCON than in E111 ($P < 0.01$) while leaf N concentrations ($P < 0.001$), root biomass ($P < 0.001$), extractable soil NO_3^- ($P < 0.2$), and N mineralization rates ($P < 0.01$) were higher (Table 3). A PCA of these 5 traits as measured on the seven species in BioCON show that correlations among these traits in BioCON were similar to the other experiment (Table 3). Species that had high leaf longevity had low leaf N concentrations, high root biomass, and low rates of N mineralization

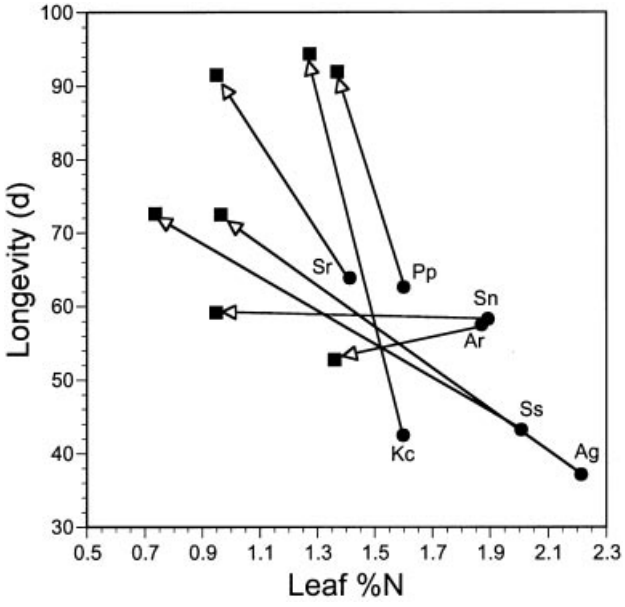

Fig. 2 N concentrations and leaf longevity of 7 species of herbaceous grassland species that were both measured in 2-yr-old, higher soil N monocultures of BioCON (closed circles) and 5-yr-old, lower soil N monocultures of Craine *et al*. (unpublished) (closed squares). Lines connect individual species between experiments. Species are denoted with a two-letter abbreviation that refers to the generic and specific epithets. Abbreviations: Kc, *Koeleria cristata*; Ag, *Andropogon gerardi*; Ss, *Schizachyrium scoparium*; Sn, *Sorghastrum nutans*; Sr, *Solidago rigida*; Ar, *Agropyron repens*; Pp, *Poa pratensis*.

(Craine *et al.*, unpublished), yet the rankings of species in the two experiments were different. There was no correlation between the two experiments in leaf longevity or leaf N concentrations of individual species (Table 3, Fig. 2). Moreover, there was a negative correlation between the two experiments in root biomass and the rates of N mineralization (Table 3) such that species that maintained the highest biomass and had the lowest rates of N mineralization after 5 yr on less productive soils, had the smallest standing biomass and highest rates of N mineralization in BioCON.

Table 3 Results of the principal components analysis of five plant and ecosystem parameters of seven species for which leaf longevity was measured in both the 5-yr-old monocultures grown on low-N soil (E111) and the BioCON experiment

	BioCON	E111	BioCON – E111	Correlation coefficient	PCA: BioCON
Leaf longevity (d)	52.2 ± 4.1	76.4 ± 6.3	−24.2**	0.02	0.45
Leaf percentageN	1.80 ± 0.10	1.08 ± 0.09	0.71***	−0.39	−0.44
Root biomass (g m^{-2})	766 ± 127	144 ± 31	622.1***	−0.75*	0.53
Soil [NO_3^-]	0.18 ± 0.04	0.10 ± 0.03	0.074	−0.44	0.32(mg g^{-1} dry soil)
Soil N mineralization (g N m^{-2})	4.62 ± 0.87	1.28 ± 0.29	3.33**	−0.85*	−0.47

Differences between the two experiments were tested with *t*-tests and the relationship of species values between the two experiments with pairwise correlations (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Discussion

All other things equal, the increase in leaf longevity due to elevated CO_2 would lead to greater ecosystem carbon gain, assuming the leaves maintained positive net photosynthesis over the additional period. Conversely, the decrease in leaf longevity due to N fertilization would lead to lower ecosystem carbon gain. Yet, CO_2 and N enrichment altered more leaf traits than longevity. The ability to scale leaf level properties to ecosystem carbon gain and productivity will require not only determining if and how CO_2 and N affect leaf longevity, but also how they affect ecosystem leaf area production and photosynthetic rates.

For example, N fertilization increased the leaf area produced by a plant over the census period and leaf photosynthetic rates (Reich *et al.*, 2001 see pp. 000–000 in this issue), which together may offset the decreases in leaf longevity that would lead to lower ecosystem carbon gain. Elevated CO_2 did not affect the leaf area production for a plant, yet increased photosynthetic rates (Reich *et al.*, 2000), which should reinforce the increases in leaf longevity for ecosystem carbon gain. Although leaf longevity is an important component of ecosystem processes, additional measurements of the onset and cessation of leaf area production and plant demography would be necessary to more reliably model the determinants of leaf area and ecosystem carbon gain.

The comparisons between BioCON and the older monocultures show the importance of ecosystem processes, as well as differences in the development of feedbacks to ecosystem processes in understanding leaf longevity. As such, understanding the increases in leaf longevity with elevated CO_2 and the decrease in leaf longevity with elevated N requires examining the effects of increased CO_2 and N supplies on ecosystem processes as well as direct ecophysiological effects on leaf longevity.

N fertilization changed leaf-level and ecosystem properties in a manner that are associated with lower leaf longevity. Photosynthetic rates, whole plant N concentrations, extractable NO_3^- and N mineralization all increased (Reich *et al.*, 2000), each associated with greater C economy of leaves and more rapid cycling of biomass and N. Still more work is necessary to understand the relationships among plant and ecosystem variables in order to understand which parameters most influence leaf area longevity and the role of leaf area longevity in determining important ecosystem parameters.

In BioCON, it appears that increases in leaf area longevity with elevated CO_2 were more congruent with declines in N mineralization and availability than changes in other leaf traits. Elevated CO_2 increased photosynthesis (Lee *et al.*, 2001 see pp. 000–000 in this issue), which is generally associated with lower leaf longevity. Elevated CO_2 did not alter SLA (Reich, unpublished), but did decrease whole plant N concentrations, extractable NO_3^- and N mineralization (Reich *et al.*, 2001), which are generally associated with greater leaf longevity.

Leaf area longevity was increased by elevated CO_2, but only for C_3 species. In another experiment, the leaf longevity of the C_4 grass *A. gerardii* increased under elevated CO_2 in open-top chambers exposed to elevated CO_2 where precipitation was restricted (Knapp *et al.*, 1999). Differences in the responses of leaf area longevity for C_4 grasses to elevated CO_2 may be associated with the degree of water stress and may be more responsive in drier years in this experiment. More work is necessary to understand the degree to which elevated CO_2 affects vegetation through changes in the N and/or water cycles (Hungate *et al.*, 1997) and how N and water availability affects plant traits such as tissue longevity.

The CO_2 response should not have been biased by pre-enrichment legacies of individual leaves. None of the leaves that we sampled were produced before the elevated CO_2 treatment began and leaves should have turned over 1.9 (*P. pratensis*, *S. rigida*) to 3.9 times (*B. gracilis*) in a 120-d growing season. Although the N treatment was applied gradually, we were able to detect both greater leaf area production and decreases in leaf area longevity under elevated N. These effects may become stronger over time as different components of the ecosystem respond at different rates and the feedbacks from CO_2 or N to leaf longevity may take longer than a growing season manifest, especially as N accumulates in the system.

In both the BioCON experiment and the 5-yr-old monocultures, there were clear differences among species in leaf longevity. Variation in leaf longevity among species in BioCON appears to reflect differences in growth rates with an N supply that is higher than that in the E111 experiment. In BioCON, it appears that species that grew the largest and fastest added more biomass to the decomposition cycle, increasing N immobilization by microbes faster and thereby decreasing N supply to plants. These plants were the ones that had the least biomass and decreased N mineralization rates the least in the less-fertile, older monocultures of E111. Although it has been stated that species growing in low-nutrient habitats have characteristics that reinforce low-nutrient availability (Hobbie, 1992), the development and the degree of trait differences need to be better examined. Leaf longevity appears to be determined at least in part by the N supply rates and therefore can be altered greatly by plant feedbacks to N cycling.

In all, it appears that comparisons of leaf longevity among species and ecosystems can not be understood without understanding nutrient cycling and species-specific development of plant-feedbacks to nutrient cycles. Responses to elevated CO_2 were species-specific and more congruent with changes in N cycling and leaf N than from the increase in photosynthetic rate. Due to the multiple pathways by which elevated CO_2 and N affect plant and ecosystem properties, better understanding of plant traits, such as leaf longevity, requires more mechanistic research that integrates for different species the direct effects of elevated CO_2 on leaf properties with effects on plant and ecosystem carbon, nitrogen, and water economy.

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