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Adjustment to the light environment in small-statured forbs as a strategy for complementary resource use in mixtures of grassland species

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• *Background and Aims* The biological mechanisms of niche complementarity allowing for a stable coexistence of a large number of species in a plant community are still poorly understood. This study investigated how small-statured forbs use environmental niches in light and CO_2 to explain their persistence in diverse temperate grasslands.
• *Methods* Light and CO_2 profiles and the corresponding leaf characteristics of seven small-statured forbs were measured in monocultures and a multi-species mixture within a biodiversity experiment (Jena Experiment) to assess their adjustment to growth conditions in the canopy.
• *Key Results* Environmental conditions near the ground varied throughout the season with a substantial CO_2 enrichment (>70 µmol mol^{-1} at 2 cm, >20 µmol mol^{-1} at 10 cm above soil surface) and a decrease in light transmittance (to <5 % deep in the canopy) with large standing biomass (>500 g d. wt m^{-2}) in the multi-species assemblage. Leaf morphology, biochemistry and physiology of small-statured forbs adjusted to low light in the mixture compared with the monocultures. However, the net carbon assimilation balance during the period of low light only compensated the costs of maintenance respiration, while CO_2 enrichment near the ground did not allow for additional carbon gain. Close correlations of leaf mass per area with changes in light availability suggested that small-statured forbs are capable of adjusting to exploit seasonal niches with better light supply for growth and to maintain the carbon metabolism for survival if light transmittance is substantially reduced in multi-species assemblages.
• *Conclusions* This study shows that adjustment to a highly dynamic light environment is most important for spatial and seasonal niche separation of small-statured forb species in regularly mown, species-rich grasslands. The utilization of short-period CO_2 enrichment developing in dense vegetation close to the ground hardly improves their carbon balance and contributes little to species segregation along environmental niche axes.

Key words: Adjustment, biodiversity, carbon dioxide, leaf mass per area, light, photosynthesis.

INTRODUCTION

The mechanisms governing community assembly and the coexistence of a large number of competing plant species in a local environment, and hence determining the diversity of the plant community, have been subject to much debate (e.g. Silvertown, 2004; Lyons *et al.*, 2005). All plants require the same resources: light, water, carbon dioxide and mineral nutrients, which they acquire in similar ways. The theory of niche differentiation assumes that differences in morphological, physiological or phenological characteristics allow for species segregation along a variety of niche axes and spatial or temporal resource partitioning among plant species (Tilman, 1997). Silvertown (2004) reported on empirical evidence for niche segregation above- and below-ground from different habitats.

Genetic differentiation of plant populations may reflect adaptation in response to their local environment (Turkington and Harper, 1979; Linhart and Grant, 1996). Apart from this, environmentally induced variation in morphological and physiological features may result in optimization of resource acquisition and species segregation in niche use. Phenotypic adjustment is particularly important in plant species because of their sessile life-form, the general inability of established plants to escape from unfavourable growth patches and their often limited capability for dispersal (Sultan, 2000; Ozinga *et al.*, 2005).

In stands of vegetation, environmental factors vary with canopy depth. The most important with respect to carbon assimilation by plants are strong gradients in light quality and quantity between upper and lower canopy layers (Monsi and Saeki, 1953). Canopy shade may also reduce vapour pressure differences between plant tissue and the surrounding air by increasing ambient humidity and decreasing temperature differences (Jones, 1992). Diurnal gradients in carbon dioxide concentration may establish within-plant canopies as a result of photosynthetic depletion, despite generation of CO_2 by plant and soil respiration, particularly under slow mixing between canopy air and the atmosphere through the boundary layer. From agricultural and forest

stands it is known that canopy CO_2 profiles are affected by leaf area index (LAI; see Table 1 for full list of abbreviations of variables) and spatial arrangement of foliage (i.e. foliage density) because wind velocity decreases exponentially within the canopy, and canopy photosynthesis increases with LAI until light, nutrients or water become limiting (Schulze *et al.*, 1994; Buchmann *et al.*, 1996; Buchmann and Ehleringer, 1998).

Light is a key limiting resource in productive environments supporting the development of dense foliage. Thus, niche partitioning along light gradients may depend on differences between species in their minimum light requirements for survival, on optimization of canopy structure for light interception, on re-acclimation to modified light environments and on phenological differences among species; all are potentially important for community structure (e.g. Kull and Niinemets, 1998; Valladares and Niinemets, 2008). Plants growing under canopy shade may respond by plasticity in a number of their traits, thus achieving higher light-harvesting efficiency in low-light environments, e.g. by increasing chlorophyll production, having a smaller leaf dry mass per unit leaf area (LMA) and less nitrogen per leaf area, and modifying shoot architecture to optimize leaf exposure and reduce self-shading (Niinemets, 2007). A greater proportion of light-harvesting apparatus (chlorophyll and chlorophyll-binding proteins) compared with electron transport and to the biochemical apparatus of photosynthesis is a typical acclimation strategy for plants growing in weak light, including canopies (Evans and Poorter, 2001). So far, differences in the capacity for light acclimation among species or functional types are not well understood and have been attributed to contrasting canopy developmental patterns and differences in the dynamics of short-term light fluctuations between herbaceous and woody canopies (Niinemets, 2007; Hallik *et al.*, 2009), rather than to structural and functional changes in the plants.

In temperate grasslands the largest fraction of community biomass usually consists of a few tall-growing dominant species (Grime, 1998; Werger *et al.*, 2002). A larger number of smaller subordinate species often only contribute

a minor portion of the total community biomass. Studies in experimental grasslands have shown that canopy height and shoot density per unit ground area increase with species richness (Spehn *et al.*, 2000; Lorentzen *et al.*, 2008). In semi-natural grasslands a tall and closed canopy is typical for productive environments with high soil fertility (Lambers *et al.*, 1990). In mixed stands of vegetation, species which are not capable of taller growth receive a disproportionally smaller share of incoming radiation because foliage of other species in the upper layers intercepts the radiation (Weiner, 1990). Above-ground competition for light increases the probability for competitive exclusion (Grime, 1973).

Increased canopy density in multi-species assemblages may also result in carbon dioxide enrichment within the canopy dependent on microclimatic factors (such as soil temperature and soil moisture) affecting soil respiration, canopy photosynthesis and respiration and boundary layer resistance for CO_2 transfer (Buchmann and Ehleringer, 1998). Higher CO_2 concentrations than ambient usually result in increasing LMA associated with higher nitrogen contents per leaf area (Poorter *et al.*, 2009). Enhanced CO_2 concentrations may stimulate photosynthesis through increasing carboxylation rates dependent on the impact of other environmental parameters such as light, temperature and humidity (Stitt, 1991). When photosynthetic photon flux density (PPFD) is such that photosynthesis can balance respiration, elevated CO_2 concentrations may shift the light compensation point to lower light intensities, allowing plants to grow in shade deeper (Körner, 2006). Therefore, it raises the question whether high CO_2 concentrations in the lower canopy can be utilized by small plants to improve their carbon gain in low light. In experiments using artificially increased CO_2 concentrations, the magnitude of growth enhancement through CO_2 fertilization was more pronounced at increasing plant species richness (Reich *et al.*, 2004), although responsiveness to elevated CO_2 in communities may be smaller than expected from individuals or populations (Wang, 2007).

Adjustment of small-statured grassland species to the light environment deep in the canopy and possible beneficial effects of natural carbon dioxide enrichment near the ground have not been experimentally tested in research on ecosystems as mechanisms of niche complementarity which affect biodiversity. In this study, using established plots of a large grassland biodiversity experiment (Jena Experiment; Roscher *et al.*, 2004), changes in the light and CO_2 environment near the ground were monitored during the growing season and morphological and ecophysiological leaf characteristics of small-statured forbs were measured in monocultures and mixture. The objectives were as follows: (*a*) to analyse whether carbon dioxide enrichment occurs in the lower canopy layers of a multi-species plant assemblage and how this correlates with changes in light availability; (*b*) to explore functional adjustments of small-statured species to optimize photosynthetic carbon gain in the lower canopy of a multi-species plant assemblage; and (*c*) to assess whether small-statured plant species benefit from this CO_2 enrichment which compensates for the weak light deeper in the canopy.

TABLE 1. *List of abbreviations of variables used in the text*

A_i	Photosynthetic rate per unit leaf area (μmol CO_2 m^{-2} s^{-1})
A_{max}	Light-saturated photosynthetic rate (μmol CO_2 m^{-2} s^{-1})
Chl	Chlorophyll concentration (mg g^{-1})
J_{max100}	Maximum electron transport rate (μmol CO_2 m^{-2} s^{-1}) at 100 μmol m^{-2} s^{-1} PPFD (low light)
$J_{max1800}$	Maximum electron transport rate (μmol CO_2 m^{-2} s^{-1}) at 1800 μmol m^{-2} s^{-1} PPFD (full light)
k	Initial slope of the light-response curve (= quantum yield, μmol CO_2 μmol photons^{-1})
LAI	Leaf area index (m^2 m^{-2})
LMA	Leaf dry mass per unit area (g m^{-2})
N_A	Leaf nitrogen per unit leaf area (g m^{-2})
N_M	Leaf nitrogen per unit leaf dry mass (mg g^{-1})
PPFD	Photosynthetic photon flux density (μmol m^{-2} s^{-1})
R_D	Dark respiration rate (μmol CO_2 m^{-2} s^{-1})
$V_{cmax100}$	Maximum carboxylation rate (μmol CO_2 m^{-2} s^{-1}) at 100 μmol m^{-2} s^{-1} PPFD (low light)
$V_{cmax1800}$	Maximum carboxylation rate (μmol CO_2 m^{-2} s^{-1}) at 1800 μmol m^{-2} s^{-1} PPFD (full light)

MATERIALS AND METHODS

Biodiversity experiment

The Jena Experiment is a large biodiversity experiment established on a former agricultural field in 2002. It is situated in the floodplain of the river Saale near to the city of Jena, Germany (50°55′N, 11°35′E, 130 m a.s.l.). The area has a mean annual air temperature of 9·3 °C and mean annual precipitation of 587 mm (Kluge and Müller-Westermeier, 2000). The 60 species in the experiment are typical of central European species-rich semi-natural grasslands (Arrhenatherion communities; Ellenberg, 1988). In addition to 78 plots, 20 × 20 m in size, with plant communities of different species richness (1, 2, 4, 8, 16) and functional group number and composition (1–4; grasses, legumes, small herbs and tall herbs), 60-species mixtures were sown with equal numbers of seeds per species. Furthermore there are monocultures of each species on smaller 3·5 × 3·5 m plots. The study presented here used a 60-species plot and a monoculture of each species investigated (see below). Plots were weeded biannually (early April and July) to remove unwanted species and mown twice a year in early June and September corresponding to the traditional management of hay meadows in the region. The field site was not fertilized during the experiment. A detailed description of the experiment is given in Roscher *et al.* (2004).

Study species

Seven small-statured forbs belonging to the functional group of 'small herbs' in the design of the Jena Experiment (Roscher *et al.*, 2004) were studied. They have in common an inability to grow above the lower canopy in multi-species assemblages of grassland species, but otherwise have different growth forms. *Bellis perennis* L. (Asteraceae), *Plantago media* L. (Plantaginaceae) and *Primula veris* L. (Primulaceae) are rosette plants that may reproduce vegetatively, forming new rosettes from axillary buds (Sagar and Harper, 1964; Schmid and Harper, 1985). *Veronica chamaedrys* L. (Scrophulariaceae) is a slightly ascending species with an initially creeping, branched rootstock that changes gradually into erect or prostrate stems that may produce adventitious roots (Harris and Lovell, 1980). *Glechoma hederacea* L. (Lamiaceae) and *Prunella vulgaris* L. (Lamiaceae) are creeping forbs; individuals grow with monopodial shoots and form root initials at nodes. Lateral meristems in leaf axils allow for the formation of higher-order stolons and a branched structure of individuals (Schmid and Harper, 1985; Slade and Hutchings, 1987). *Ajuga reptans* L. (Lamiaceae) usually forms a rosette that may develop into a flowering stem. Lateral stolons grow out from the base in different directions dependent on the position of axillary buds. Stolons have adventitious roots at each node and may form orthotropic shoots after a period of plagiotropic growth (Dong *et al.*, 2002).

Canopy characteristics

Carbon dioxide and water vapour concentrations in the canopy air were monitored in vertical profiles from different heights within and above the canopy (2, 10, 20 and 30 cm each with three replicates, 50 cm with two replicates, and 180 cm, resulting in a total of 15 sample points) in a 60-species plot during the growing seasons 2007 and 2008. From each point, air was sampled with a membrane pump (KNF Neuberger GmbH, Freiburg, Germany) for 60 s, completing the whole system within 15 min. CO_2 and water vapour were measured each second after a 10-s break with an infrared CO_2/H_2O gas analyser (LI-6262; LI-COR, Lincoln, NE, USA) when the system had been switched to the next sample point. Readings of each sample point were averaged and stored with a datalogger (CR10X; Campbell Scientific, Logan, UT, USA). The system was calibrated biweekly. In addition, PPFD was measured in vertical profiles with five replicates at a height of 2, 10 and 20 cm and three replicates at a height of 30 and 50 cm. PAR (photosynthetically active radiation) sensors were based on a photodiod (BPW21) covered by a diffuse plastic disc and calibrated against a quantum sensor (Q22543; LI-COR). Voltage was measured at 10-s intervals, resulting in 90 readings for an average value per 15 min. Sensors were multiplexed through an AM416 (Campbell Scientific) and data were stored with a second datalogger (CR10X). Air temperature and relative humidity (HMP35D humidity/temperature sensor; Vantaa, Vaisala, Finland) and PAR (PAR LITE; Kipp & Zonen, Delft, The Netherlands) at a height of 2 m were measured at a central weather station at the field site and stored with a datalogger (CR23X; Campbell Scientific).

Above-ground plant biomass was determined by harvesting one 1·0 × 1·0 m plot in strata (>75 cm, 50–75 cm, 35–50 cm, 25–35 cm, 15–25 cm, 5–15 cm, <5 cm) in the 60-species mixture at estimated peak biomass immediately before mowing in early June and September 2007 and 2008. Plant material was weighed after drying for 48 h at 70 °C to get dry mass.

Plant measurements

Leaf gas exchange was measured with a LI-6400 portable photosynthesis system equipped with a 6400-02B LED light source (LI-COR) in August 2007. Firstly, A/C_i curves were recorded at low-light (100 μmol m^{-2} s^{-1}) and at full-light conditions (1800 μmol m^{-2} s^{-1}), each starting at ambient concentrations of 400 μmol mol^{-1} CO_2 followed by a step-wise reduction of CO_2 concentrations to 300, 200, 100 and 50 μmol mol^{-1} CO_2, returning to ambient concentrations of 400 μmol mol^{-1} CO_2, and increasing step-wise to 500, 600, 900, 1200, 1700 and 2200 μmol mol^{-1} CO_2 in the leaf chamber. Secondly light-response curves were measured at constant CO_2 of 380 μmol mol^{-1} at 1800, 1500, 1000, 900, 800, 600, 400, 300, 100, 50, 25 and 0 μmol m^{-2} s^{-1}. Leaf temperature was kept constant at 25 °C. Records were taken when the sample leaf was equilibrated for at least 60 s under each CO_2 and light level. All measurements were made between 20 and 26 August 2007. Measurements were replicated on three fully expanded leaves per species belonging to different individuals in the respective monocultures and the 60-species mixture. Before starting leaf gas exchange measurements, the highest point of foliage above soil surface of the respective shoots and canopy height of the surrounding vegetation were measured to assess species positioning within the canopy. After termination of

all measurements the part of the leaf enclosed in the leaf chamber was cut off and its area was determined by drawing the contour on graph paper. After drying (70 °C, 48 h) leaf samples were weighed to calculate LMA. Leaf material was ground to a fine powder and analysed for nitrogen concentrations with an elemental analyser (Vario EL element analyser; Elementar, Hanau, Germany). Light-response curves served to calculate light-saturated photosynthetic rate (A_{max}; μmol CO_2 m^{-2} s^{-1}), dark respiration rate (R_D; μmol CO_2 m^{-2} s^{-1}) and quantum yield (k; μmol CO_2 μmol photons^{-1}) using the hyperbolic tangent function by Jassby and Platt (1976). Maximum carboxylase activity of Rubisco (V_{cmax}; μmol CO_2 m^{-2} s^{-1}) and the capacity for photosynthetic electron transport (J_{max}; μmol CO_2 m^{-2} s^{-1}) were assessed from A/C_i curves at low and high light availability according to Harley and Tenhunen (1991). Additional light-response curves at constant CO_2 of 380 μmol mol^{-1} and 500 μmol mol^{-1}, respectively, at 400, 300, 200, 100, 50, 25 and 0 μmol m^{-2} s^{-1} were measured between the 6 and 7 June 2008 at estimated peak biomass with three replicates (leaves) per species in the mixture.

Material of three fully expanded leaves originating from different plants was collected shortly before mowing on 2 September 2007. Each leaf sample was divided into two parts. After leaf area determination using graph paper, the first sub-sample was dried at 70 °C (48 h) and weighed. Ground leaf material was analysed for nitrogen concentrations (see above). The second sub-sample was stored in the dark at −80 °C until sample preparation. Chlorophyll a and b concentrations (Chl$a$ and Chl$b$) were determined in 80 % acetone extracts measuring absorbances at 647 and 664 nm with a UV-Vis spectrophotometer (Helios Gamma; Thermo Spectronic, Erlangen, Germany) following Ziegler and Egle (1965).

A bulk sample of leaves (youngest expanded leaves from three shoots) of each species in monoculture and the mixture was collected biweekly during the growing season 2008 (between 21 April and 25 August) to assess seasonal variation in leaf morphology in response to different growth conditions. After transport in a cool box to the laboratory, the area of each leaf sample was determined with an LI-3100 area meter (LI-COR). Dry mass (after 48 h at 70 °C) was used to calculate LMA.

Data analyses

Statistical analyses were performed with the software R2.6.2 (R Development Core Team; http://www.R-project.org). A two-way ANOVA based on the factors growth environment (monoculture vs. mixture), species identity and their interaction, was used to test for effects on leaf ecophysiological characteristics determined in August 2007. Tukey's test was utilized for multiple pairwise comparisons of sample means per species. Measurements of photosynthetic rates at different CO_2 concentrations in the 60-species mixture in May 2008 were explored with repeated-measures ANOVA. Mixed-effects models using the *lme* function implemented in the *nlme* package of the statistical software R (Pinheiro and Bates, 2000) were applied for analysis of seasonal variation

of LMA to account for unbalanced replicates due to single missing samples. Plot identity was introduced as random effect. A series of models was fitted including growth environment (monoculture vs. mixture), species identity, sampling date and interaction terms among these factors as fixed effect using the maximum likelihood method. Likelihood ratio tests (= L ratio) served to compare models and to estimate the significance of the fixed effects. A standardized principal components analysis (PCA) using CANOCO 4·5 (ter Braak and Šmilauer, 2002) was performed to explore relationships among leaf characteristics measured in August 2007. Leaf characteristics were averaged per species and growth environment for PCA.

Furthermore, field-derived leaf physiological characteristics of the different species were used to model net carbon gain for a 10-d period (20–30 August 2007) using data from CO_2 and light profiles. For this purpose, a leaf-scale photosynthesis and respiration model developed by von Stamm (1994) was used. In contrast to the widely used Farquhar model (Farquhar *et al.*, 1980; Farquhar and von Caemmerer, 1982), this model works more empirically and calculates stomatal conductance, independently from internal CO_2, from photosynthesis rate. This allows for an independent modelling of stomatal acclimation to water relations as also described by Kutsch *et al.* (2001, 2008). Leaf CO_2 exchange depends on stomatal conductance, light, temperature and leaf properties derived from the field measurements. Acclimation of the photosynthetic apparatus to shade was included as decreases in photosynthetic capacity (A_{max}) and dark respiration, and an increase in the initial slope of the light curve, characterized by an empirical coefficient (k). Response to CO_2 was calculated by a linear increase that was observed between 350 and 600 μmol mol^{-1} in the A/C_i curves. To assess whether CO_2 enrichment deep in the canopy (2 and 10 cm above soil surface) may result in an increased carbon gain compared with the CO_2 environment above the canopy, scenarios with ambient (measured above the canopy) and elevated carbon dioxide (measured within the canopy) at low light were compared. In addition, monoculture and mixture leaves in the mixture growth environment were modelled to assess whether leaves adjusted to the growth conditions in a mixture achieve a higher carbon gain than leaves adjusted to growth conditions in monoculture.

RESULTS

Canopy characteristics in a 60-species mixture and species positioning within the canopy

CO_2 concentrations and light availability varied strongly with season and re-growth after mowing (Fig. 1), but values at peak biomass before mowing were dependent on standing biomass associated with different canopy density and height (Fig. 2A, B). Average CO_2 enrichment compared with that above the canopy was 105 μmol mol^{-1} at 2 cm, and 23 μmol mol^{-1} at 10 cm above the soil surface in late May 2008 before first mowing, and was 74 μmol mol^{-1} at 2 cm, and 20 μmol mol^{-1} at 10 cm above the soil surface in August 2007 before second mowing (average of quarter-hourly measurements from 1000 to 1400 h in a 2-week period before
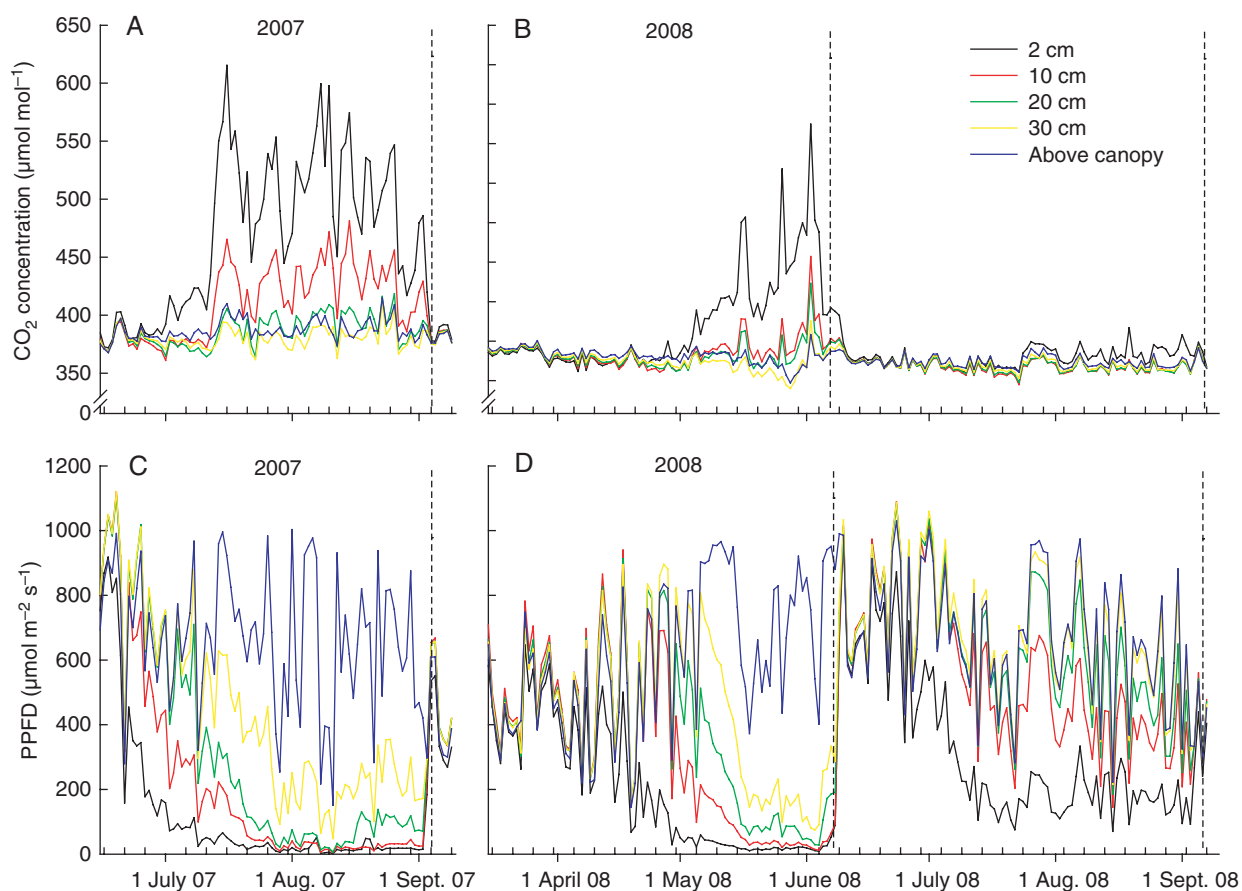
FIG. 1. Seasonal course of canopy profile CO_2 concentrations in (A) 2007 and (B) 2008, and photosynthetic photon flux density (PPFD) in (C) 2007, and (D) 2008 in a 60-species mixture 2 cm, 10 cm, 20 cm and 30 cm and 180 cm (200 cm, respectively, for PPFD) above the soil surface. Shown are daily averages of half-hourly automatic measurements based on a time frame where PPFD $\geq 100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ above the canopy (200 cm height). Vertical dashed lines indicate the time of mowing.

mowing; Fig. 2C). Carbon dioxide enrichment at a height of 2 cm was much lower (5 μmol mol^{-1}) and did not occur in higher strata of the canopy profile in August 2008 when above-ground biomass was 250 g d. wt m^{-2}, while above-ground biomass harvested in late August 2007 and early June 2008 was >500 g d. wt m^{-2} (Fig. 2A–C). The upper canopy layers were composed of tall grasses (*Alopecurus pratensis*, *Arrhenatherum elatius*, *Dactylis glomerata*, *Trisetum flaves-cens*) and forbs (*Knautia arvensis*, *Medicago × varia*, *Onobrychis viciifolia*). At the time of greatest canopy density, before mowing in late August 2007 and May 2008, PPFD measured above the canopy was reduced to <3 % and <5 %, respectively, at 2 cm and 10 cm above soil surface (Fig. 2D). PPFD decreased only to approx. 33 % at 2 cm and 68 % at 10 cm above soil surface in August 2008 when stand-ing biomass was 50 % lower than in August 2007 and May 2008.

Average height of foliage differed significantly between the species studied in the mixture at the time of peak biomass in August 2007 (Table 2), ranging from 4 cm (*A. reptans*) to 11 cm (*P. veris*). Height of foliage varied between monoculture and mixture depending on species (Table 2); it was significantly higher in *V. chamaedrys* in the mixture than in the monoculture (Table 3).

Nevertheless, foliage of the small species only reached 5–14 % of the height of the surrounding vegetation (Tables 2 and 3).

Leaf morphology and biochemistry in monoculture vs. mixture

LMA, leaf nitrogen per unit area (N_A) and leaf nitrogen per unit dry mass (N_M) differed among species (Table 2). All species had leaves with a lower LMA in the mixture than in the monoculture (Fig. 3A), while differences in N_M and N_A between monoculture and mixture were species specific (Table 2). N_A of *A. reptans* and *P. vulgaris* were significantly lower in the mixture than in the monoculture (Fig. 3B), while N_M of one species (*G. hederacea*) increased in the mixture (Fig. 3C).

Although chlorophyll concentrations varied between mono-cultures and the mixture depending on species identity (Table 2), they were significantly higher in the mixture of all species with the exception of *A. reptans* (Fig. 3D). Chl$a$: Chl$b$ and Chl/N ratios (Table 2) also differed among species. Decreases in Chl$a$: Chl$b$ from monocultures to the mixture were species specific and significant for *P. media* and *P. veris* (Fig. 3E). Although Chl/N ratios varied depending on species identity, all species except for *B. perennis* had
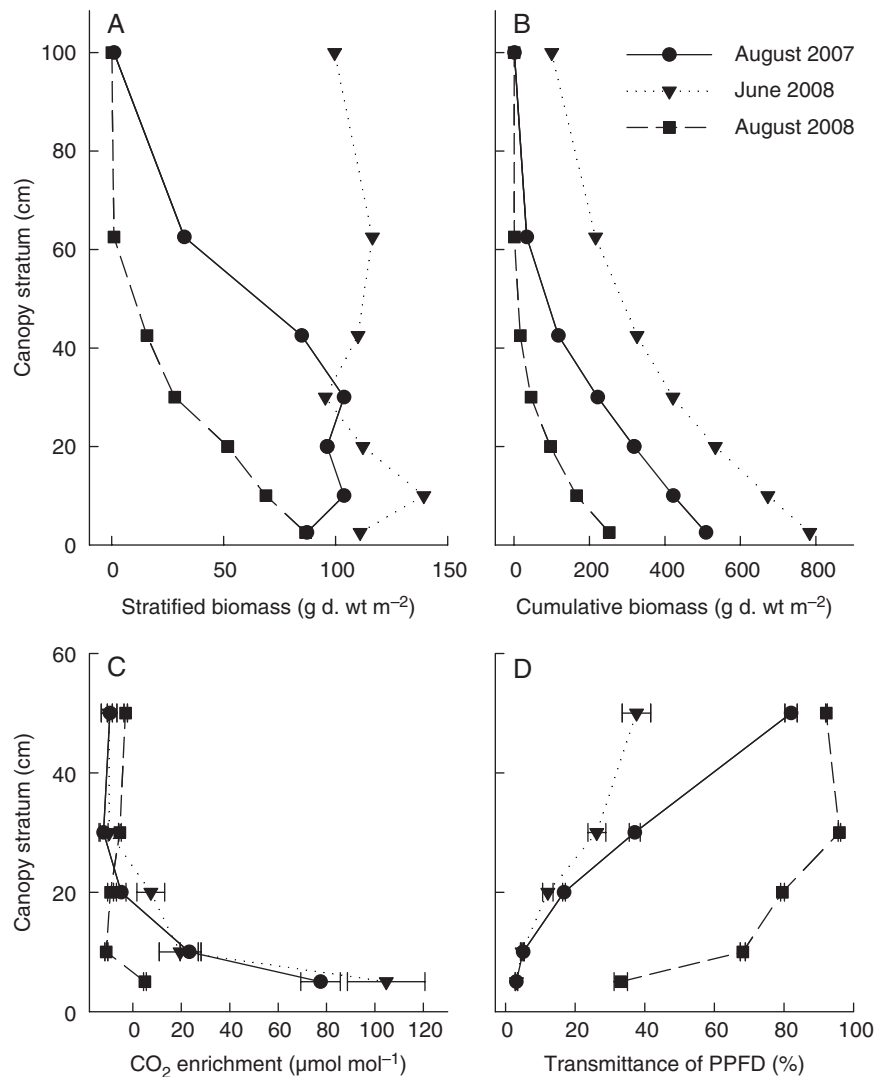
FIG. 2. (A) Biomass (dry mass), (B) cumulative biomass, (C) CO_2 enrichment (compared with CO_2 concentrations at 180 cm above ground), (D) and proportional transmittance of PPFD (compared with full-light conditions at 200 cm above ground) plotted against canopy strata in August 2007, June 2008 and August 2008 shortly before mowing in a 60-species mixture. Light transmittance and CO_2 enrichment were derived from daily means ($\pm$ s.e.) of quarter-hourly averages at midday (1000–1400 h) across a 2-week period at peak canopy density before mowing.

higher Chl/N ratios in the mixture than in their monocultures (Fig. 3F).

Leaf gas exchange in monoculture vs. mixture

The A_{max} per unit area of leaf did not vary significantly among species and was higher in monocultures than in the mixture (Table 2). Differences in A_{max} between monoculture and the mixture were only statistically significant for *P. vulgaris* (Fig. 4A). The R_D per unit leaf area varied among species and was generally larger in monoculture, but differences between monoculture and mixture were only significant for *G. hederacea* (Fig. 4B and Table 2). A_{max} and R_D per unit leaf mass were unaltered between monoculture and mixture, but differed among species (Tables 2 and 3). Initial quantum yield (k) was not different among species or between growth environments (Fig. 4C and Table 2). Leaf area-based $J_{max1800}$ and $V_{cmax1800}$ at full-light conditions of

1800 μmol m^{-2} s^{-1} did not differ among species, but were lower in the mixture (Table 2). However, the difference in $J_{max1800}$ and $V_{cmax1800}$ between monoculture and mixture was only significant for two species, *G. hederacea* and *P. vulgaris* (Fig. 4D, E). There were no statistically significant differences between species and growth environment in area-based J_{max100} and $V_{cmax100}$ at 100 μmol m^{-2} s^{-1} (Fig. 4F, G). Leaf mass-based $J_{max1800}$ and $V_{cmax1800}$ at full-light conditions did not differ between growth environments, while mass-based J_{max100} and $V_{cmax100}$ at low-light conditions were significantly larger in the mixture (Tables 2 and 3).

Relationships between combinations of leaf traits in monocultures and mixture

The first axis of a standardized PCA of leaf characteristics explained about 57 % of variance and separated monoculture leaves from mixture leaves (Fig. 5). This axis had high positive

TABLE 2. *Summary of two-factorial ANOVA of leaf ecophysiological characteristics of seven small-statured forbs measured in monocultures (= Mono) and a 60-species mixture (= Mix) in August 2007 shortly before second mowing*

Leaf characteristics	Mono vs. Mix (plot) F	Mono vs. Mix (plot) P		Species identity (ID) F	Species identity (ID) P	ID × plot F	ID × plot P
Height of foliage	**13·90**	**0·001**	↑	**9·45**	**<0·001**	**3·73**	**0·008**
Height of foliage relative to canopy height	**31597·36**	**<0·001**	↓	**4·61**	**0·002**	**4·81**	**0·002**
Leaf dry mass per unit area (g m^{-2})	**286·56**	**<0·001**	↓	**5·29**	**0·001**	**2·93**	**0·024**
Leaf nitrogen per unit area (g m^{-2})	**106·60**	**<0·001**	↓	**10·68**	**<0·001**	**4·41**	**0·003**
Leaf nitrogen per unit mass (mg g^{-1})	**8·75**	**0·006**	↑	**4·59**	**0·002**	**7·34**	**<0·001**
Chlorophyll (mg g^{-1})	**216·08**	**<0·001**	↑	1·36	0·265	**6·35**	**<0·001**
Chlorophyll *a/b*	**92·75**	**<0·001**	↓	**4·31**	**0·003**	1·61	0·183
Chlorophyll/N ratio	**302·46**	**<0·001**	↑	**6·64**	**<0·001**	**3·74**	**0·007**
Area-based A_{max} (μmol CO_2 m^{-2} s^{-1})	**39·84**	**<0·001**	↓	1·30	0·290	0·85	0·544
Area-based R_D (μmol CO_2 m^{-2} s^{-1})	**21·98**	**<0·001**	↓	**3·80**	**0·007**	1·43	0·238
k (μmol CO_2 μmol photons^{-1})	0·33	0·572		0·65	0·686	1·21	0·330
Area-based $J_{max1800}$ (μmol m^{-2} s^{-1})	**81·91**	**<0·001**	↓	0·62	0·712	1·33	0·280
Area-based $V_{cmax1800}$ (μmol m^{-2} s^{-1})	**52·28**	**<0·001**	↓	0·31	0·926	1·15	0·364
Area-based J_{max100} (μmol m^{-2} s^{-1})	0·09	0·769		1·51	0·212	0·41	0·866
Area-based $V_{cmax100}$ (μmol m^{-2} s^{-1})	0·23	0·637		0·75	0·618	0·30	0·931
Mass-based A_{max-m} (μmol CO_2 g^{-1} s^{-1})	0·14	0·706		**5·19**	**0·001**	0·83	0·561
Mass-based R_D (μmol CO_2 mg^{-1} s^{-1})	0·54	0·471		**5·53**	**0·001**	1·29	0·296
Mass-based $J_{max-m1800}$ (μmol g^{-1} s^{-1})	0·79	0·382		1·78	0·142	0·79	0·585
Mass-based $V_{cmax-m1800}$ (μmol g^{-1} s^{-1})	0·93	0·097		1·33	0·279	1·71	0·157
Mass-based $J_{max-m100}$ (μmol g^{-1} s^{-1})	**42·34**	**<0·001**	↑	**2·66**	**0·037**	0·58	0·742
Mass-based $V_{cmax-m100}$ (μmol g^{-1} s^{-1})	**35·14**	**<0·001**	↑	1·79	0·140	0·50	0·805

Significant effects of growth environment (plot), species identity (ID) and interactions between plot × ID are given in bold.
Arrows indicate increases (↑) or decreases (↓) in mixture compared with monocultures.

loadings for LMA, N_A, Chl*a* : *b*, A_{max}, $J_{max1800}$ and $V_{cmax1800}$ at full-light and R_D (characterizing monoculture leaves) and high negative loadings for chlorophyll concentrations and Chl/N ratios (characterizing mixture leaves). The second axis explained about 18 % of variance. It correlated positively with J_{max100} and $V_{cmax100}$ at low-light conditions, R_D and k. Positive loadings correlated with the position of *V. chamaedrys* in both environments, negative loadings characterized *P. vulgaris* in both environments, while other species showed a larger variation in these traits between monoculture and the mixture (e.g. *A. reptans*, *G. hederacea*).

Effects of mixture growth environment on carbon gain

Light-response curves measured at constant CO_2 of 380 μmol mol^{-1} and 500 μmol mol^{-1} shortly before mowing in June 2008 using three independent leaves per species in the mixture indicated a positive CO_2 effect on photosynthetic uptake rates at low-light conditions of 50 μmol photons m^{-2} s^{-1} ($F_{1,14} = 11·83$, $P = 0·004$), while this effect was not statistically significant when light intensity was reduced to 25 μmol photons m^{-2} s^{-1} ($F_{1,14} = 3·24$, $P = 0·094$; Fig. 6).

Model calculations for a 10-d period at an estimated peak biomass (20–30 August 2007) showed that mean net assimilation across species amounted to $0·00 \pm 0·61$ μmol CO_2 m^{-2} s^{-1} at a height of 2 cm and $1·78 \pm 0·76$ μmol CO_2 m^{-2} s^{-1} at a height of 10 cm above ground under measured intra-canopy CO_2 conditions in the mixture compared with net assimilation under CO_2 conditions above the canopy ($-0·06 \pm 0·57$ μmol CO_2 m^{-2} s^{-1} at a height of 2 cm; $1·72 \pm 0·73$ μmol CO_2 m^{-2} s^{-1} at a height of 10 cm). Intra-canopy CO_2 enrichment did not increase carbon gain of the species studied (Tukey's test, $P > 0·050$). Additional calculations showed that the carbon gain of leaves adjusted to growth conditions in the mixture was not significantly higher than that for leaves adjusted to monoculture growth conditions modelled under the mixture growth environment (Tukey's test, $P > 0·050$).

Seasonal variation of leaf morphology

Repeated determination of LMA in 2008 showed significant variation throughout the growing season ($L = 76·50$, $P < 0·001$), with a significant difference between monocultures and the mixture ($L = 5·47$, $P = 0·018$; Fig. 7). Seasonal variation of LMA depended on species identity ($L = 166·76$, $P < 0·001$). The coefficient of variation (CV) of LMA in the mixture was more than twice that in monoculture for *G. hederacea* and *P. vulgaris*, while the variation in LMA was only slightly different between both growth environments in the other small forbs. In all species, except for *P. veris* ($r_S = 0·357$, $P = 0·388$, $n = 7$) and *P. media* ($r_S = 0·617$, $P = 0·067$, $n = 9$), LMA in the mixture correlated positively with the average daily PPFD at 10 cm above ground level ($r_S \geq 0·650$, $P < 0·050$; PPFD = average of 5 d before the leaf harvest) indicating an adjustment to seasonal changes in light climate. Seasonal variation of LMA in leaves growing in monoculture under full light did not correlate with the average daily PPFD above the canopy.

DISCUSSION

The use of different ecological niches is crucial for the coexistence of different species in a local environment, although

TABLE 3. *Height of foliage and leaf mass-based ecophysiological characteristics of small-statured forb species measured in monocultures (Mono) and a 60-species mixture (Mix) in August 2007 shortly before second mowing*

		A. reptans		B. perennis		G. hederacea		P. media		P. veris		P. vulgaris		V. chamaedrys	
Shoot height (cm)	Mono	5.0 ± 0.0		6.0 ± 0.0		5.0 ± 0.0		6.0 ± 0.0		8.0 ± 0.0		5.0 ± 0.0		5.3 ± 0.3	
	Mix	4.3 ± 0.3		5.3 ± 0.3		6.7 ± 0.3		6.7 ± 0.3		10.5 ± 2.5		7.7 ± 1.5		9.0 ± 0.6	$*\uparrow$
Relative height to the canopy (%)	Mono	100 ± 0		100 ± 0		100 ± 0		100 ± 0		100 ± 0		100 ± 0		100 ± 0	
	Mix	5 ± 0	$***\downarrow$	9 ± 1	$***\downarrow$	10 ± 1	$***\downarrow$	10 ± 1	$***\downarrow$	13 ± 3	$***\downarrow$	11 ± 2	$***\downarrow$	14 ± 2	$***\downarrow$
Mass-based A_{max-m} (μmol CO_2 g^{-1} s^{-1})	Mono	0.16 ± 0.03		0.36 ± 0.04		0.29 ± 0.03		0.19 ± 0.02		0.17 ± 0.02		0.25 ± 0.05		0.22 ± 0.00	
	Mix	0.19 ± 0.02		0.32 ± 0.03		0.26 ± 0.03		0.26 ± 0.04		0.15 ± 0.00		0.23 ± 0.01		0.28 ± 0.08	
Mass-based R_D (μmol CO_2 mg^{-1} s^{-1})	Mono	-8.3 ± 2.1		-31.8 ± 8.8		-14.4 ± 10.9		-17.1 ± 2.1		-18.2 ± 1.3		-20.7 ± 5.3		-43.2 ± 5.6	
	Mix	-16.8 ± 10.8		-43.7 ± 2.2		-31.7 ± 8.3		-15.7 ± 3.0		-17.8 ± 5.2		-20.6 ± 1.0		-33.4 ± 4.1	
Mass-based $J_{max-m1800}$ (μmol g^{-1} s^{-1})	Mono	20.5 ± 2.1		35.9 ± 3.6		33.7 ± 5.4		19.4 ± 1.6		19.9 ± 1.6		26.6 ± 4.1		29.8 ± 4.1	
	Mix	16.9 ± 3.1		34.0 ± 5.2		21.7 ± 1.2		25.0 ± 2.1		19.6 ± 4.6		21.6 ± 1.0		19.7 ± 11.6	
Mass-based $V_{cmax-m1800}$ (μmol g^{-1} s^{-1})	Mono	11.1 ± 1.1		25.6 ± 5.0		22.8 ± 4.6		13.4 ± 1.3		14.3 ± 2.5		15.8 ± 3.4		21.1 ± 4.8	
	Mix	17.1 ± 9.0		16.3 ± 3.2		13.8 ± 0.6		15.5 ± 1.6		10.8 ± 2.0		11.6 ± 0.8		13.0 ± 3.0	
Mass-based $J_{max-m100}$ (μmol g^{-1} s^{-1})	Mono	6.2 ± 0.6		11.0 ± 0.1		8.6 ± 1.0		5.2 ± 0.5		6.9 ± 0.6		5.9 ± 1.4		10.0 ± 1.1	
	Mix	11.8 ± 2.8		19.1 ± 1.9		14.0 ± 0.4		12.8 ± 1.5		9.7 ± 1.1		15.2 ± 0.8		20.0 ± 3.7	
Mass-based $V_{cmax-m100}$ (μmol g^{-1} s^{-1})	Mono	3.8 ± 0.3		6.2 ± 0.3		5.1 ± 0.9		2.6 ± 0.5		3.6 ± 0.3		3.1 ± 0.7		5.2 ± 1.0	
	Mix	7.2 ± 2.2		8.8 ± 1.7		9.0 ± 0.7		7.1 ± 1.1		5.7 ± 1.1		8.3 ± 0.6		11.2 ± 2.9	

Values are means ($\pm$ s.e.).
Tukey's test was applied to test for differences between monoculture and mixture values, where $* P \leq 0.05$, $** P < 0.01$ and $*** P < 0.001$.
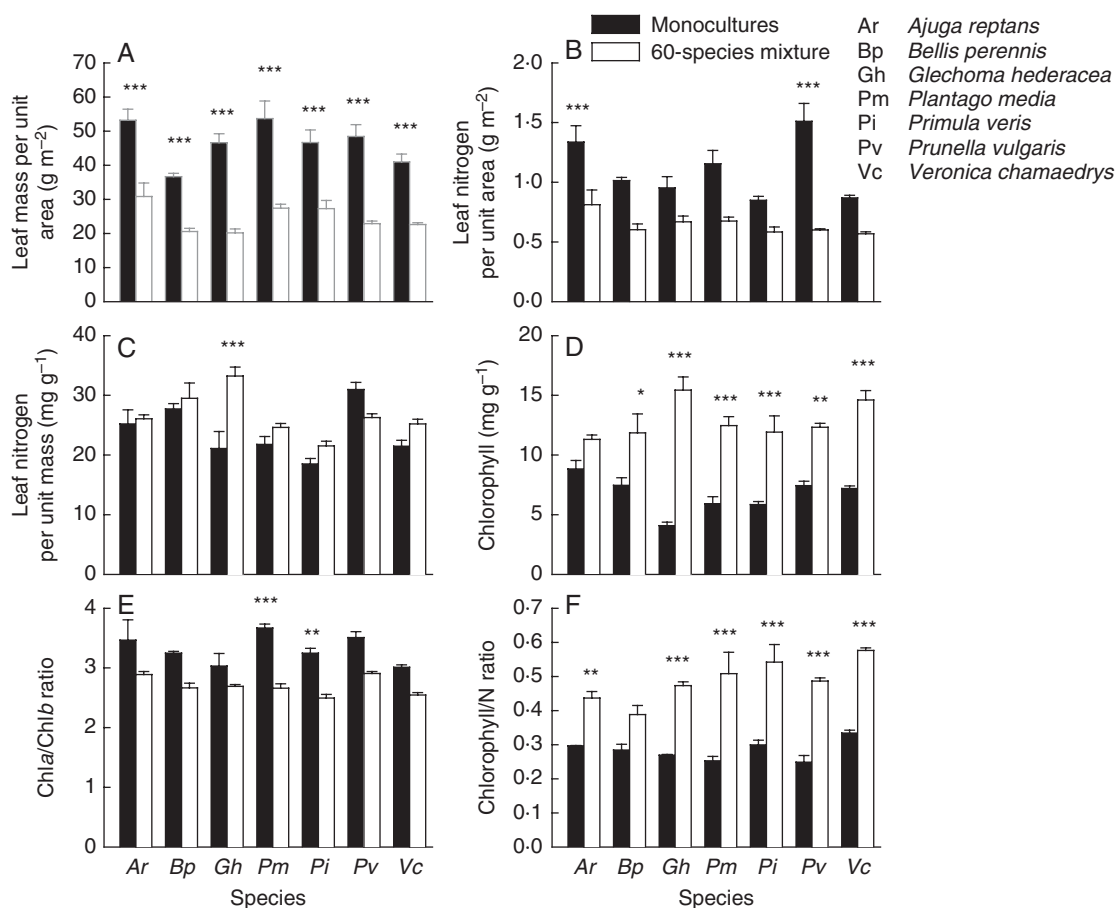Arrows indicate larger values in mixture ($\uparrow$) or monoculture ($\downarrow$).

FIG. 3. (A) Leaf dry mass per unit area, (B) leaf nitrogen per unit area, (C) leaf nitrogen per unit dry mass, (D) chlorophyll concentration, (E) Chl*a* : Chl*b* ratio and (F) chlorophyll/N ratio of leaves harvested in early September 2007 shortly before second mowing in species monocultures and a 60-species mixture. All values are means ($\pm$ s.e.) from three independent leaf samples. Tukey's test was applied to test for differences between monoculture and mixture values, where * $P \leq 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Abbreviations: *Ar*, *Ajuga reptans*; *Bp*, *Bellis perennis*; *Gh*, *Glechoma hederacea*; *Pm*, *Plantago media*; *Pi*, *Primula veris*; *Pv*, *Prunella vulgaris*; *Vc*, *Veronica chamaedrys*.

there is little obvious potential for separation of resource niches among plant species that all depend on a common set of resources. Specialization in particular regions of niche axes in which it is superior to competitors, determines a species' survival and reproduction. Niche complementarity increasing total resource use at the community scale has also been suggested as a mechanism to explain the positive relationship between species richness and above-ground productivity observed in a number of biodiversity experiments (Hooper *et al.*, 2005). Carbon assimilation in plants varies with CO_2 concentration and light availability. In this study, continuous measurements of canopy CO_2 profiles were combined in a 60-species mixture with measurements of morphological and physiological leaf traits in monocultures and mixture to analyse the performance of small-statured forbs during periods of high light competition. The species under investigation on average achieve a higher or equal above-ground biomass in mixtures than expected from their monoculture biomass production (Marquard *et al.*, 2009), but so far the mechanisms that enable these species to survive and reach a positive carbon balance in multi-species mixtures are not well understood.

One hypothesis is that these species benefit from higher CO_2 concentrations close to the surface. The present study shows carbon dioxide enrichment in the deepest canopy of the 60-species plot, which is probably a consequence of increased soil microbial activities with increasing plant species richness (Eisenhauer *et al.*, 2010). This result contrasts with that of Caldeira *et al.* (2001) in Mediterranean experimental grasslands, where only very small differences in CO_2 concentrations near the ground were observed, attributable to low canopy densities even in communities with higher plant diversity. Generally, the magnitude of intra-canopy gradients of CO_2 increase with increasing LAI due to increased boundary layer resistance for CO_2 transfer in dense canopies (Baldocchi *et al.*, 1981). This is in line with the present results, where a noticeable CO_2 enrichment correlated with a decreasing light transmittance in lower canopy layers when foliage became denser, and it did not occur in late August 2008 when above-ground biomass in the mixture reached only 50 % (approx. 250 g d. wt m^{-2}) of biomass harvested in early June 2008 and late August 2007 shortly before mowing. Lower productivity in August 2008 was probably due to low precipitation in the summer months (3-month
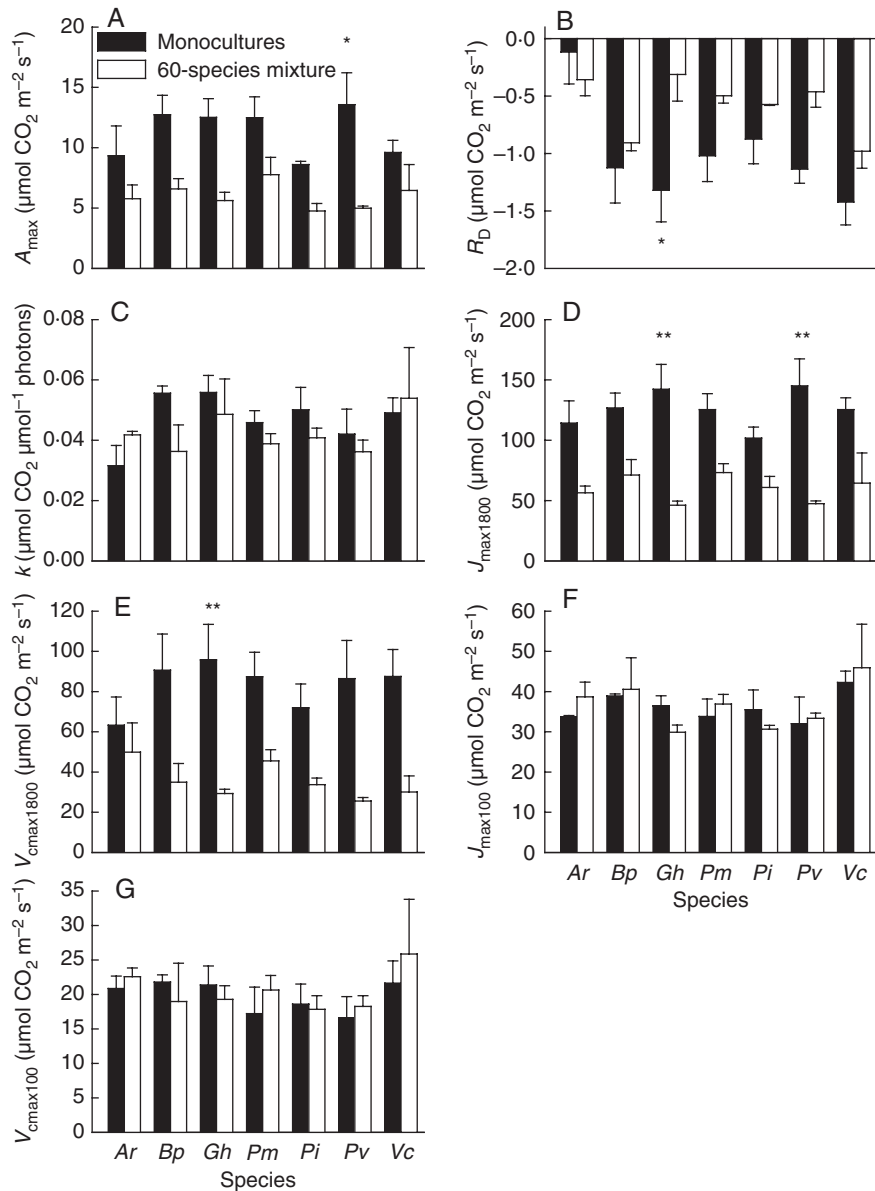
FIG. 4. (A) Light-saturated photosynthetic rate A_{max}, (B) initial quantum yield k, (C) dark respiration rate R_D, (D) maximum electron transport rate $J_{max1800}$ at full light of 1800 μmol m^{-2} s^{-1}, (E) maximum carboxylation rate $V_{cmax1800}$ at full light, (F) J_{max100} at low light of 100 μmol m^{-2} s^{-1}, and (G) $V_{cmax100}$ at low light of leaves measured in August 2007 in species monocultures and a 60-species mixture. All values are means ($\pm$ s.e.) from measurements of three independent leaves. Tukey's test was applied to test for differences between monoculture and mixture values, where * $P \leq 0.05$, ** $P < 0.01$ and *** $P < 0.001$. For abbreviations of species' names see Fig. 3.

sum from June to August = 154 mm) compared with summer 2007 (3-month sum = 238 mm).

The continuous measurement of available photosynthetic active radiation in the canopy profile of the mixture showed substantial variation in light climate across the growing season and the effects of mowing and succeeding re-growth (Fig. 1). Plant species respond to this highly variable niche with respect to light availability in different ways. Small-statured species not able to emerge above the canopy shade may avoid temporal niches with poor light availability or adjust to this variation (Schulze and Chapin, 1987; Hirose and Werger, 1995). Increased internode and petiole lengths, stem weight and leaf area ratios and reduced branching have

been observed under low photon flux density for a number of small-statured grassland species including *G. hederacea* and *V. chamaedrys* (Corré, 1983; Slade and Hutchings, 1987; Dale and Causton, 1992*a*). For species with inherent growth restrictions which cannot position their leaves above the leaves of other species, regardless of shoot morphological adjustments (Hutchings and de Kroon, 1994), physiological adjustment becomes particularly important. Studies in natural grasslands have indicated that inter-specific differences in carbon gain are more closely related to leaf physiological than to shoot morphological differences (Anten and Hirose, 2003; Aan *et al.*, 2006). Increases in total Chl and Ch 1 : N ratios and decreasing Chl*a* : Chl*b* under reduced illumination
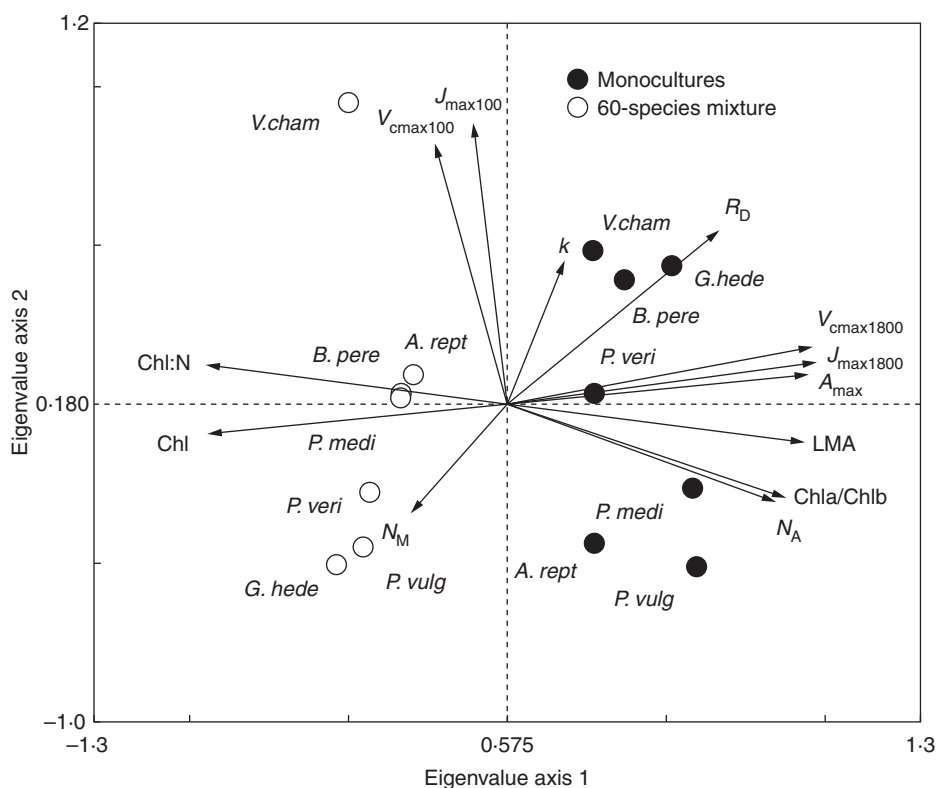
FIG. 5. Standardized principal components analysis (PCA; first vs. second axes) of leaf characteristics of seven small-statured forbs measured in monocultures and a 60-species mixture in August 2007. Abbreviations of species names are: *A.rept, Ajuga reptans*; *B.pere, Bellis perennis*; *G.hede, Glechoma hederacea*; *P.medi, Plantago media*; *P.veri, Primula veris*; *P.vulg, Prunella vulgaris*; *V.cham, Veronica chamaedrys*; for names of variables see Table 1.
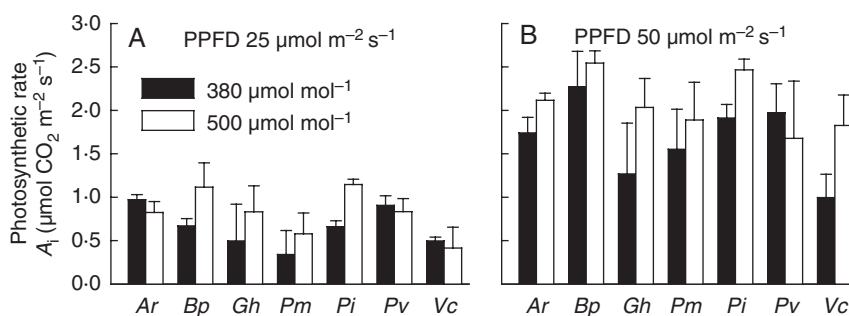

FIG. 6. Photosynthetic rates (A_i) at photosynthetic photon flux density (PPFD) of 25 μmol m^{-2} s^{-1} (A) and 50 μmol m^{-2} s^{-1} (B) based on light curves measured at constant CO_2 of 380 μmol mol^{-1} and 500 μmol mol^{-1}, respectively, in June 2008 at peak biomass in a 60-species mixture. Values are means ($\pm$ s.e.) of three independent leaves per species. For abbreviations of species' names see Fig. 3.

are adaptive responses to increase light harvesting (Dale and Causton, 1992*b*; Kull and Niinemets, 1998), which were also observed in our comparison of leaves from the mixture and monocultures. This acclimation to shade represents a shift in the relative importance of resource investment from carbon-fixing components to those responsible for light harvesting, which is often reflected in N partitioning among them (Hikosaka and Terashima, 1996; Evans and Poorter, 2001). Furthermore, high rates of photosynthesis are associated with higher costs for maintenance of larger concentrations of photo-synthetic enzymes (Valladares and Niinemets, 2008) and the superior performance of shade-tolerant species has been repeatedly explained by their capacity to minimize CO_2

losses rather than by maximizing their potential carbon gain (Walters and Reich, 2000; Craine and Reich, 2005). Thus, a decrease in photosynthetic capacity results in smaller dark res-piration rates and in a lower light compensation point. Most of these trait syndromes indicative of shade tolerance were observed in the comparative analysis. Leaf area-based A_{max}, $J_{max1800}$ and $V_{cmax1800}$ at full-light conditions and R_D were lower when small-statured forbs grew in the mixture as opposed to monocultures. These differences were mostly attributable to the formation of leaves with a smaller LMA in the mixture and did not differ significantly per unit leaf dry mass (see Table 2). This shows that the combination between structural (e.g. variation in LMA) and biochemical
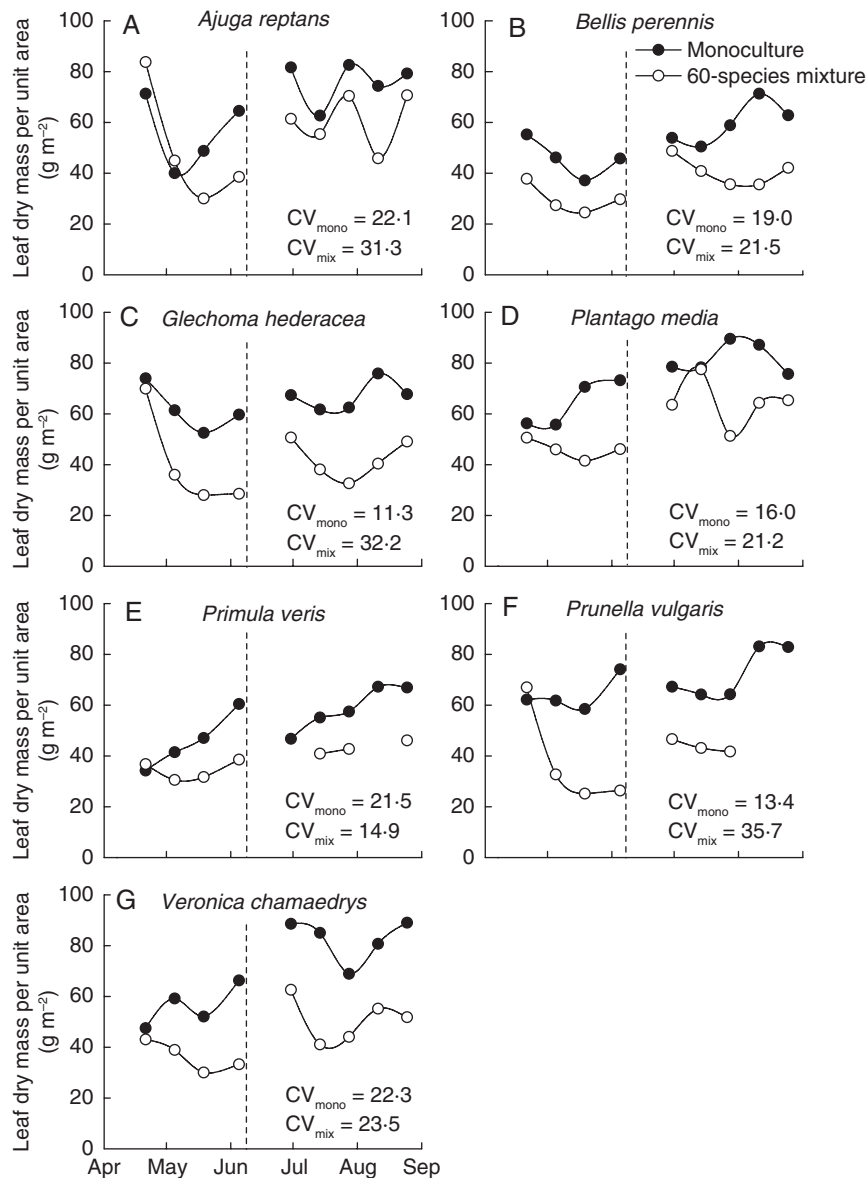
FIG. 7. Seasonal variation of leaf dry mass per area (LMA) of (A) *Ajuga reptans*, (B) *Bellis perennis*, (C) *Glechoma hederacea*, (D) *Plantago media*, (E) *Primula veris*, (F) *Prunella vulgaris* and (G) *Veronica chamaedrys* in monocultures and in a 60-species mixture measured biweekly during the growing season 2008. Vertical dashed lines indicate time of the first mowing. CV_{mono} = coefficient of variation of LMA in monoculture through season, CV_{mix} = coefficient of variation of LMA in mixture through season.

(e.g. changes in Chl) leaf traits is essential for acclimation to different light climates, which was confirmed by the multivariate analysis of leaf characteristics (Fig. 5) and is in line with other studies (e.g. Ellsworth and Reich, 1993; Poorter *et al.*, 2006). Interestingly, J_{max100} and $V_{cmax100}$ measured under low-light conditions did not differ among monoculture and mixture leaves indicating an optimal allocation between light capture and carbon fixation in the mixture environment. Although a small increase in photosynthetic rates was found at higher light intensities when CO_2 concentrations in the leaf chamber were increased from 380 to 500 μmol CO_2 m^{-2} s^{-1} (Fig. 6), modelling of carbon assimilation in response to different light and carbon dioxide supplies showed that, despite the acclimation, the carbon balance of small-statured

forbs was close to zero when the canopy was fully developed. Data and modelling indicated clearly that the plants only benefit negligibly from the increased CO_2 concentration at low light. Their adjustment is targeting to low light acclimation and is not an acclimation to increased CO_2 concentrations.

Light availability below the plant canopy typically declines during the course of the growing season, as LAI is chiefly determined by growth of taller, dominant species. Consequently, growth of subordinate species early in the season or immediately after mowing may increase their light acquisition by escaping the substantial impact of tall species (Anten and Hirose, 1999). Carbon gain early in the season and immediately after mowing when the vegetation is short

enough for a better light supply are probably most important for the long-term carbon balance of small-statured forbs. Studies of leaf carbohydrates of two small-statured forbs (*P. media* and *V. chamaedrys*) in the middle of May and in July after first mowing showing that increasing species richness did not influence daily carbon turnover (Daßler *et al.*, 2008) and metabolite profiling of leaf material collected at peak biomass before first mowing of another small-statured forb (*B. perennis*) indicating carbon and nitrogen limitation at increasing species richness (Scherling *et al.*, 2010) support this explanation.

Hallik *et al.* (2009) showed that acclimation patterns in specific leaf area (inverse of LMA), leaf nitrogen and chlorophyll concentrations through vertical light gradients are poorly developed in herbaceous vegetation compared with deciduous tree canopies. In deciduous tree canopies, leaves develop at beginning of the growing season from perennial branches, and therefore the light environment of an individual leaf is more constant during its lifespan (Niinemets *et al.*, 2004). In contrast, in herbaceous vegetation stems and leaves are continuously produced during canopy development and major changes in the light envionment may occur during the lifespan of individual leaves (Anten *et al.*, 1998; Terashima *et al.*, 2005). The present seasonal analysis of LMA in a mixture (Fig. 7) indicated that small-statured forbs have a remarkable capacity to adjust the morphology of newly developing leaves in accordance with changing light environments during the growing season. The only species, *P. veris*, where no correlation between LMA and seasonal changes in light availability near the ground was observed, reaches its phenological maximum in spring and, depending on weather conditions, its leaves become senescent in summer. The other small forb species, except for *P. media*, remain all-season with foliage (Rothmaler, 2002).

Conclusions

This study provided clear evidence that small-statured forbs in mown grassland can only compensate the costs of maintenance during the period of low light when tall species intercept the radiation, while short-term CO_2 enrichment close to the ground hardly improves the carbon gain and did not substantially compensate for decreased light. As photosynthetic adjustment of leaves to higher CO_2 concentrations may be costly and time-consuming, the small-statured forbs investigated are obviously not selected to benefit from the temporary CO_2 enrichment that may occur at high canopy density. The small-statured forbs investigated in this study typically grow in regularly mown temperate grasslands, while their population sizes often decline or the species even undergo extinction after abandonment (e.g. Blomqvist *et al.*, 2003; Johansson *et al.*, 2011). Large seasonal variation in leaf morphology indicated that the growth strategy of small-statured forbs is adjusted to exploit seasonal niches with better light supply for growth and to maintain carbon metabolism for survival if light transmittance in multi-species assemblages is reduced to low levels. The adjustment to highly dynamic niches in light availability enables small-statured forbs to coexist with taller neighbours in regularly mown diverse grassland communities, but increases the probability of their extinction with the cessation of management and the development of a dense litter layer. Further studies on the use of seasonal niches are required to understand ecological differences among co-existing small-statured forbs and their contribution to ecosystem functioning.

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