Plasticity of leaf and shoot morphology and leaf photochemistry for *Brachypodium pinnatum* (L.) Beauv. growing in contrasting microenvironments in a semiarid loess forest-steppe vegetation mosaic

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**Summary**

After clearcutting xerothermic oakwoods once natural in semiarid temperate loess regions of Hungary the perennial understorey grass *Brachypodium pinnatum* (L.) Beauv. may persist through decades and often dominates grasslands maintained by grazing and/or cutting in the place of former oakwoods. This grass also successfully establishes from low- to high-light microenvironments co-occurring as forest regeneration commences after pasture abandonment. It was assumed that *B. pinnatum* must possess a high degree of phenotypic plasticity for such an ecological versatility. This assumption was tested by comparing leaf and shoot morphology and leaf photochemistry in the species’ three typical microenvironments (full shade under oak canopy, half shade near shrubs, and full sun in unshaded grassland) for plants growing *in situ* and for those reciprocally transplanted between these microhabitats. Aboveground standing crop of *B. pinnatum* was greatest near shrubs, indicating that in this microhabitat light limitation (typical in oak shade) and water stress (appearing temporarily in the grassland) are ameliorated. Average leaf lifespan was greatest under oak canopy, while leaf senescence was highest in the grassland. An efficient adjustment to low light was observed both in leaf morphology (specific leaf mass, leaf thickness and bulk tissue density were lowest in oak shade) and in leaf photochemistry (quantum yield of PSII (ΦPSII), photochemical quenching (qP), and non-photochemical energy dissipation (NPQ) were lower, while PSII antenna efficiency (Fv'/Fm') was higher for leaves in oak shade than for others). Transplanted plants showed remarkable phenotypic plasticity since after one year of transfer their leaves did not differ in photochemistry and/or morphology from those growing *in situ* in the new microenvironment. However, transplants appeared to be more sensitive to the high radiation load in the grassland than *in situ* ones. Our results confirm the high capacity of *B. pinnatum* for phenotypical adjustment to habitat light environment, that is consistent with the species’ original forest-steppe coenological affinity and also may contribute to the species’ persistence after deforestation. Nevertheless, temporary water stress associated with high radiation load in the unshaded grassland appear to pose a limitation on the ecological distribution of this species in Central Europe.

Key words: phenotypic plasticity, sun and shade leaves, specific leaf mass, phenology, transplant experiment.

Abbreviations: D = leaf bulk tissue density; ETR = relative electron transport rate; Fv/Fm = PSII maximum photochemical efficiency; Fv'/Fm' = antenna efficiency of PSII; LAI = leaf area index; NDVI = leaf normalized difference vegetation index; NPQ = non-photochemical fluorescence quenching; qP = photochemical fluorescence quenching; PPFD = photosynthetic photon flux density; PRI = photochemical reflectance index; REIP = red edge inflection point; SLM = specific leaf mass; T = leaf thickness; VAZ pool = violaxanthin-antheraxanthin-zeaxanthin cycle pigment pool; ΦPSII = PSII actual quantum yield at a given irradiance

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Introduction

*Brachypodium pinnatum* (L.) Beauv. is a perennial, rhizomatous grass with an extensive root system and erect shoots. In humid regions of Western and Central Europe it is a characteristic element of seminatural species-rich chalk grasslands, which had been grazed throughout centuries. Most recently an increasing dominance of *B. pinnatum* in these grasslands was recognized – even beyond the northern limit of this species’ range in an experimental study (Buckland et al. 2001) – at the expense of sympatric plant species, and was ascribed to elevated nitrogen deposition, abandonment and change from grazing to autumn mowing since World War II (Bobbink & Willems 1987, 1988). Therefore, the success of this grass poses a serious threat to species diversity, particularly to the abundance of forbs of low stature and short-lived species (Bobbink & Willems 1987; Hurst & John 1999a). In Hungary, under the semiarid temperate climate of the Carpathian Basin, *B. pinnatum* dominated grasslands are also mainly of secondary origin, developed after deforestation in various vegetation zones and on several bedrock types, although few primary types were also recognized (Schmotzer & Vojtkó 1996). Originally *B. pinnatum* was a characteristic understorey species in open xerothermic oakwoods which intermingled with steppe grasslands. These were once typical communities in loess areas of the Hungarian Great Plain and neighbouring foothills as part of the forest steppe vegetation zone (Zólyomi & Fekete 1994). In historic times most of these oakwoods have been cut, steppe grasslands were turned into arable fields. Natural vegetation was able to survive only in small, isolated fragments on slopes too steep for cultivation or on undisturbed prehistoric earthworks (Zólyomi & Fekete 1994; Zólyomi 1969). After cutting the oakwoods the understory grass *B. pinnatum* usually survives for decades. It even becomes dominant in the developing xero-mesic grassland if forest regeneration is suppressed by grazing or cutting. Furthermore, it also successfully establishes from shade to full sun microhabitats mostly on slopes facing N to E, but rarely occurs on slopes facing S or SW. In semiarid areas of Hungary *B. pinnatum* does not appear to possess such an invasive behaviour as in the humid alpine regions of Europe (Bobbink & Willems 1987). *B. pinnatum* grasslands in Hungary possess a remarkably high species diversity preserving numerous elements of the former oakwood, and thus have a great nature conservation value (Virág & Bartha 1998; Fekete et al. 1998). However, during a secondary succession after deforestation *B. pinnatum* grasslands may gradually turn into xeric steppe grasslands dominated by *Festuca rupicola*, a sclerophyllous grass of wide ecological distribution in the Carpathian Basin (Fekete et al. 1998; Zólyomi & Fekete 1994). Cessation of traditional land use, such as grazing or mowing, poses another threat for these species-rich grasslands since certain shrubs (e.g. *Crataegus monogyna*) rapidly colonize them as an early phase of forest regeneration. Since the propagules of the original climax species (oaks) are hardly available in the region, dense thickets may stabilize and the grassland species will unable to persist in the deep shade.

Substantial knowledge has already accumulated on *B. pinnatum* concerning its clonal morphology, growth characteristics, biomass and nutrient allocation patterns, and the plastic responses of these traits to different levels of light intensity and nutrient availability compared with other sympatric calcareous grassland species (De Kroon & Knops 1990a,b,c; Poorter & Remkes 1990; Ryser & Lambers 1995). *B. pinnatum* has a moderate relative growth rate (Poorter & Remkes 1990), clones with phalanx growth form and characteristics of the consolidation strategy (de Kroon & Schieving 1990), and was classified as a stress tolerant competitor according to Grime (1979). Field experiments so far mainly focused on the effects of additional nutrient (particularly nitrogen) supply on above- and below-ground phytomass of *B. pinnatum* and other species in chalk grasslands. When only N was added, *B. pinnatum* became dominant (Buckland et al. 2001; Bobbink 1991) mostly due to its internal nutrient cycling mechanism (Bobbink et al. 1989), and a good phosphorous acquisition capacity (Bobbink 1991), although short-term glasshouse and garden growth experiments revealed not such an efficient phosphorus economy relative to other sympatric grass species (Ryser & Lambers 1995; Ryser et al. 1997). At high nutrient availability, however, when both N and P was increased, faster growing nutrient demanding grasses (e.g. *Arrhenatherum elatius*, *Dactyliis glomerata*, *Holeus lanatus*) outperformed *B. pinnatum* (Buckland et al. 2001; Bobbink 1991). Changes in vegetation structure caused by an increased shoot mass of *B. pinnatum* – resulting in a decrease in light availability – are considered to be the main cause of restricted performance of many short-lived or prostrate species under elevated N supply (Bobbink et al. 1988; Bobbink 1991; Neitzke 2001). Hurst & John (1999a) found higher soil nitrate and lower light level under *B. pinnatum* stands than under surrounding vegetation. Mowing in mid-summer, sheep grazing or using the herbicide glyphosate proved to be adequate management to reduce the success of *B. pinnatum* (Bobbink & Willems 1988; Bobbink 1989; Hurst & John 1999b), however the reduction of soil nutrient levels should also be a part of a management regime for the maintenance of species-rich chalk grassland communities (Hurst & John 1999a,b; Neitzke 2001).

Rather than focusing on the influence of nutrient enrichment, that is possibly less important in the rela-
tively nutrient-rich loess vegetation than in a nutrient-limited chalk grassland, in this study we aimed at to explore the physiological, phenological and morphological responses of *B. pinnatum* to different microenvironments in a vegetation mosaic developed as a consequence of deforestation and subsequent secondary succession. It was assumed, that high plasticity of these traits may contribute to the persistence or only gradual replacement of this species without the protection by the forest overstorey. First, we examine the variation of *B. pinnatum* plants growing *in situ* in three typical microhabitats (under oak canopy (shade), near shrubs in grassland (half-shade), and in the grassland exposed to full sun). At the canopy level we studied leaf area index (LAI) and above ground phytomass (standing crop), at the individual level shoot morphology and phenology, and at the leaf level photochemical, morphological and structural parameters. Second, in a field experiment we transplanted plants among microhabitats to test the hypothesis that this species possesses a substantial plasticity in the above traits.

**Materials and methods**

**Study area**

The study site is located on a steep, NE-facing slope in a valley near the village Isaszeg 25 km east of Budapest at an altitude of 200–230 m a.s.l. at the border of the Gödöllő Hills, Hungary. The area is within the forest-steppe vegetation zone. The climate is transitional between the submediterranean-continental type climate of the Hungarian Great Plain and the subcontinental climate of the hilly regions of the Hungarian Central Range. Annual mean temperature is 9 °C, yearly precipitation is about 600 mm. Brown forest soil of chernozem character is typical on the loess substrate (Fekete et al. 2000). The site was formerly covered by open, xerothermic *Quercus pubescens* oakwood with *B. pinnatum* in the understorey. However, most of the forest was cut in the early 1900’s. Today, a mosaic of small groups of remaining oaks with *B. pinnatum* in the herb layer, *Crataegus monogyna* shrub patches, and a series of grassland communities occur along the NE slopes of the valley. In the past decade the area was not grazed or cut. According to a hypothetical scheme for secondary succession (Fekete et al. 2000) at the time scale of decades the xeromesic *B. pinnatum* community, which had expanded at numerous places along the slope after deforestation, gradually turns into xeric grasslands. Thus various types of *B. pinnatum* grasslands appear in close proximity nowadays from dense and closed swards along the edge of *C. monogyna* shrubs – which represent an early phase of secondary succession and preserve numerous forest and forest-steppe plant species – through transitional types to the so called steppe type *B. pinnatum*-community, which is not influenced by the shade of shrubs or remnant trees (Fekete et al. 2000; Virág & Bartha 1998). This steppe type grassland represents a late successional stage, where the percentage cover of *B. pinnatum* declines, its vitality is low with leaves yellowing more often than in other types, whereas the abundance of *Festuca rupicola* and many other steppe species becomes considerable.

**Experimental design**

To test the plasticity of certain plant traits for *B. pinnatum* a field experiment was set up using the following seven treatments (Fig. 1): plants growing *in situ* 1) under the oak tree canopy (“Tree”), 2) near *C. monogyna* shrubs (“Shrub”), or 3) in the unshaded grassland (“Grassland”); and plants transplanted from below the oak canopy 4) to the exposed grassland (“Tree-Grassland”) or 5) into the close vicinity of shrubs (“Shrub-Grassland”), and from the edge of shrubs 6) to the exposed grassland (“Shrub-Grassland”) and 7) to the oak subcanopy (“Shrub-Tree”). Plants were transplanted in May 1999 in the following way. Fifteen monoliths containing the sward and soil were dug out from *B. pinnatum* communities both in the oak subcanopy (shade) and shrub edge (half-shade) microhabitats, potted into PVC plastic tubes of 25 cm diameter and 20 cm depth, and than divided into three groups of 5 samples. One group from each of these two microhabitats was reciprocally transplanted, another group was transferred into the grassland (full sun) microhabitat, while the third group was used as control and left *in situ*. One shoot per monolith was chosen for morphological and physiological measurements. By using plastic tubes for the control treatment as well we intended to minimize the effect of experimental manipulation on the results, with the exception of the grassland *in situ* treatment, where no plastic tubes were applied. This was so because *B. pinnatum* was present in very low abundance in the grassland microhabitat at the date of transplanting. The bottom of plastic tubes were left open and the side wall were per-
for all the following variables were calculated for each microhabitat: total aboveground standing crop, stem dry mass and leaf dry mass per unit soil surface area (g m⁻²), proportion of foliage and stem in shoot dry mass (%), and specific leaf mass (SLM, leaf dry mass per unit leaf area, g m⁻²)

Leaf area index (LAI, m² m⁻²) was estimated by using the following expression: (total leaf area per ten shoots x leaf dry mass per sample plot of 0.25 m² x 4)/leaf dry mass per ten shoots.

**Shoot morphology and leaf phenology**

On 20 April 2001 five *B. pinnatum* shoots per treatment were chosen for phenological observations and labelled at stem base with a small ring of plastic tape. These shoots were inspected every other week until 14 June, and then once in every three weeks until 18 October. All labelled shoots remained vegetative and did not produce flowers throughout the study period. Number of leaves and phenological stages of each leaf blade per shoot were recorded on each day of data collection. For leaves three different phenological stages were distinguished:

- **a)** newly emerged leaves with involuted blade; **b)** leaves unfolded but not yet fully expanded; and **c)** fully expanded leaves with exposed ligule.

Length and width (measured 1–1.5 cm from the attachment of leaf blade and sheath) of not fully and fully-expanded leaves were measured until leaves reached their final length. From the onset of senescence the length of the senescent part of each leaf blade was also recorded. Each tips of newly emerged leaves were marked by using permanent marker pens of different colour at the date of their first observation.

Leaf surface area was underestimated by regarding leaf blades as triangles and using the expression of length × width/2 for calculation. Therefore, after measuring the length and width of three leaf blades of other (not the labelled) shoots per treatment and calculating their area, leaves were cut and the area of these leaves was measured with an LI-3000A leaf area meter (LI-COR Inc., Lincoln, Nebraska) in the laboratory as well. The ratio between measured and calculated area was averaged for each treatment, and leaf surface area of labelled shoots was corrected by using these factors. Total leaf area per shoot was calculated by adding the corrected area of single fully and not fully-expanded leaves per shoot together. (Number and surface area of folded leaves per shoot were neglected.) Number of fully and not completely expanded leaves per shoot, total leaf area per shoot, senescence percentage of total leaf area per shoot and size of individual fully-expanded leaves were averaged for the five (in the case of *Grassland plants* 4 and *Tree-Grassland plants* 3) shoots per treatment on each day of sampling (1 labelled shoots of *Grassland plants* and 2 ones of *Tree-Grassland plants* died early in the growing season during the study). Leaf longevity was estimated for the period from the folded stage until the full senescence of 1–6 leaves per shoot and averaged for treatments.

**Data collection**

**Microclimate**

In the three typical microhabitats (oak shade, shrub edge and grassland) air temperature was automatically measured and recorded in every 10 minutes from mid June to the end of August 2001 by using three HOBO Pro Temp sensors (Onset Computers Inc., Bourne, MA, USA). The measurement period could not be longer since the limited availability of equipment. Measurement accuracy was 1°C. Sensors were protected from rain and direct solar radiation, and were positioned 20–30 cm above the soil surface within the bulk of *B. pinnatum* canopy. Midday (11.00–12.00 h) photosynthetic photon flux density (PPFD) in the PAR spectrum (400–700nm) was measured above *B. pinnatum* shoots labelled for morphological and phenological observations between May (after full development of oak canopy) and August on cloud-free days by using an EMS-7 Canopy Transmission Meter (PP-Systems, Hitchin, U.K.). Volumetric soil water content in the top 6 cm of soil was measured near labelled shoots (in the case of transplanted plants and Shrub plants in the middle and out of the plastic tubes as well) from April until October on each date of leaf phenological data collection by using a ThetaProbe ML2 Soil Moisture Sensor (Delta-T Devices, Cambridge, UK). For each date average soil water content per treatment (n = 5) was calculated. Daily precipitation and daily mean temperature data for the years 2000 and 2001 were obtained from the meteorological station of the Hungarian Meteorological Service at the city Gödöllő ca. 8 km from our study site.

**Grass canopy measurements: leaf area index (LAI) and aboveground standing crop**

In July 2000 three plots of 0.25 m² were laid out in a homogeneous *B. pinnatum* dominated sward in each microhabitat. Shoots were clipped at soil surface and transported to laboratory in plastic bags. Ten shoots per plot were arbitrarily chosen, where the number of leaves per shoot were determined and leaves were detached from stems. Total one-sided surface area of leaves with 0.1 cm² accuracy was measured by using an LI-3000A leaf area meter (LI-COR Inc., Lincoln, Nebraska). Stem thickness was measured above the lowermost node with a precision of 0.01 mm by using a thickness meter (Mitutoyo, Japan), and internode length was measured between two nodes using a ruler (except for stems possessing only one node). All plant material in samples was separated into leaves and stems, dried in an oven at 80°C for 3 hours, then weight-ed. From these data the following variables were calculated for each microhabitat: total aboveground standing crop, stem dry mass and leaf dry mass per unit soil surface area (g m⁻²), proportion of foliage and stem in shoot dry mass (%), and specific leaf mass (SLM, leaf dry mass per unit leaf area, g m⁻²)

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Chlorophyll a fluorescence induction

Photochemical responses of leaves to PPFD were studied by using a pulse-modulated chlorophyll a fluorimeter (Hansatech Fluorescence Monitoring System FMS2, Hansatech Instruments Ltd., Norfolk, England) on the youngest fully-expanded leaves of three shoots of Tree, Shrub-Tree, Shrub and Grassland plants in April (n = 3) and of five shoots of each treatment in May and June 2000 (n = 5). After 15 min dark incubation maximum photochemical efficiency \((\Phi_l = (Fv/Fm) \times qP)\) was determined applying a saturating light pulse (0.7 s, ca. 4000 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\)), then a light response curve was generated using the halogen actinic light source of the fluorimeter delivered to the leaf by an optical fiber. Leaves were exposed to actinic light from 16 to 1650 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) in ten steps. Leaves were incubated for 20 s under each light intensity, then light-incubated chlorophyll fluorescence parameters were determined after Schreiber et al. (1986) as follows: \(Fv'/Fm' = (Fm' - Fo')/(Fm' -Fs)\), \(\Phi PSII = Fv'/Fm' \times qP\), \(ETR = \text{PPFD} \times 0.5 \times 0.84 \times \Phi PSII\), \(NPQ = (Fm' - Fm)/Fm'\). The short incubation time (20 s) was chosen so that measurements with this sample size could be made within a relatively short time (between 10.00 and 14.00 h) and to avoid substantial diurnal changes in chlorophyll fluorescence parameters common under field conditions (see e.g. Björkman & Demmig-Adams 1994; Demmig-Adams & Adams 1996). As a consequence of the short incubation time, at low light intensities photosynthesis of the leaves was not fully in steady-state. At high irradiance levels this problem did not occur, therefore we analysed chlorophyll fluorescence parameters obtained at the highest incubation PPFD (1650 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)). Nevertheless, with cautions such “instant light response curves” with not fully steady-state conditions may be used for intraspecific comparisons (Rascher et al. 2000; Hamerlynck 2001).

Leaf spectral reflectance

Parallel to chlorophyll a fluorescence induction measurements leaf spectral reflectance was also recorded. This non-invasive technique allows rapid estimation of leaf photosynthetic – chlorophyll and xanthophyll cycle – pigment content by analysing the spectral composition of light reflected from the leaf. Several spectral indices have been developed, which are derived from the reflectance spectrum and are proportional to certain pigment components (see Gamon & Surfus 1999; Sims & Gamon 2002 for review). After calibration against wet analytical techniques, leaf spectral reflectance may allow the determination of absolute pigment contents. However, we did not go so far in the analysis and compared reflectance indices – as measures of relative pigment content – instead of absolute pigment concentrations, an approach sufficiently reliable in intraspecific comparisons (Sims & Gamon 2002). Leaf spectral reflectance was measured in the 300–1100 nm wavelength range with approximately 3 nm nominal bandwidth by using an UNISPEC field portable spectrometer (PPSystems, Haverhill, MA, USA). After field measurements leaves were detached, immersed with their cut surface in tap water and dark incubated overnight in humid air at room temperature in the laboratory. Next day the reflectance spectrum was recorded on the dark-incubated sample, then the leaf was illuminated with high intensity actinic light, and reflectance was recorded in every 30 s until a stable reading was obtained (usually within ten minutes). This procedure allows the estimation of the VAZ pool size in leaves as described by Gamon & Surfus (1999).

The following reflectance indices were calculated after Sims & Gamon (2002): leaf normalized difference vegetation index \((\text{NDVI} = (R_{\text{700}} - R_{\text{760}})/(R_{\text{700}} + R_{\text{760}}))\) and red edge inflection point \((\text{REIP, defined as the wavelength of maximum slope in the red - near infrared region of the reflected spectrum, } \text{REIP} = \Lambda_{\text{max}}(\text{REIP}))\), both estimating leaf chlorophyll content, and photochemical reflectance index \((\text{PRI} = (R_{531} - R_{570})/(R_{531} + R_{570}))\) showing the relative amount of de-epoxidized (photoprotective) form of xanthophyll cycle pigments. \(R_{\text{max}}\) in formulas refers to leaf reflectance at wavelength nnn in nanometers. Total VAZ pool size was estimated as \(\Delta\text{PRI} = \text{PRI}_{\text{dark}} - \text{PRI}_{\text{light}}\) where \(\text{PRI}_{\text{dark}}\) and \(\text{PRI}_{\text{light}}\) are the PRI values for dark- and light-incubated leaves, respectively. Both leaf optical techniques (chlorophyll fluorescence and spectral reflectance measurements) were applied on the same leaves (n = 5) throughout with spectral reflectance measurement always preceding leaf dark incubation and subsequent chlorophyll a fluorescence measurement. Leaf spectral reflectance was recorded on fully developed leaves, except in September and October 2000 for the Grassland and Shrub treatments when it was done on young leaves of newly emerged shoots (n = 5). \(\Delta\text{PRI}\) was measured in the year 2000 only.

**Leaf coarse structure**

In April, May and June 2000 the youngest fully-developed leaves of five shoots per treatment were collected and transported to the laboratory in humid chambers. The one-sided surface area, thickness near the central vein (T, mm) and dry mass were measured, and specific leaf mass (dry mass per unit leaf area, SLm, g m\(^{-2}\)) and leaf bulk tissue density (dry mass per unit leaf volume, \(D = \text{SLm}/T\), g cm\(^{-3}\)) were calculated.

**Statistical analysis**

Two-way ANOVA with treatments and months as grouping variables was used with subsequent least significant difference (LSD) test (Sokal & Rohlf 1981) to analyse significant differences among means of chlorophyll fluorescence, leaf spectral reflectance and leaf structural parameters by using the Statistica 4.5 program package (StatSoft Inc., Tulsa, USA). For variables that fulfilled the two assumptions of ANOVA (normality and homoscedasticity) only within the same month or within the same treatment, one-way ANOVA and LSD test were used for comparisons of means among treatments or among months, respectively. Significant differences in leaf spectral reflectance parameters between May and June 2001 within the same treatment were tested by using the unpaired t-test. One-way ANOVA and LSD test were used to analyse the effect of microhabitat type on means of morphological attributes measured at shoot level (n = 30) and at canopy level...
(n = 3). When data did not meet the assumptions of ANOVA the Kruskal-Wallis test with Dunn’s post hoc test was applied for multiple comparisons using the Graphpad Software (San Diego, USA). Linear (Pearson) correlation was used to analyze the relationship between certain chlorophyll fluorescence (Fv'/Fm’, NPQ) and leaf spectral reflectance (APRI) parameters. Means of volumetric soil water content measured in the middle and outside the plastic tubes were compared by using a paired t-test, and as differences were significant only for four sampling dates (in the case of one treatment each, data not shown), the effect of plastic tubes on soil water status can be neglected. Significant differences in the estimated leaf longevity, in soil water content before the leaf production of oaks in April, after the full development of oak canopy in May, in a dry period of June and after several rainy days in July among the 7 treatments, as well as in seasonal midday PPFD across 5 treatments (the three treatment in the grassland were not distinguished) were tested with the Kruskal-Wallis test with Dunn’s test. Differences were considered significant at p < 0.05 level in each statistics.

Results

Microclimate

The weather during the two years of our study was not similar. The late spring and early summer of 2000 was exceptionally hot and dry since monthly precipitation reached only 16% and 13.3% of the long term average, while monthly mean temperature exceeded the multiyear average with 1 and 1.5 °C in May and June, respectively. The temporal distribution of rainfall was even more unfavourable as a continuum of 30 days passed without precipitation between mid April and mid May. In contrast, in 2001 rainfall was 42% and 105% of the long term average in May and June, respectively. From mid June through late August 2001 daily mean air temperature within the B. pinnatum sward was similar near the shrubs and under the oak tree canopy (between 13–24 °C), but was usually 1–2 °C higher in the grassland. Within-sward maximum air temperature increased in the direction of oak canopy < shrubs < grassland microhabitats. Daily temperature fluctuation averaged 19.4 °C in the grassland that was about 5 °C and 7 °C higher than that near the shrubs and under the oak tree, respectively. On bright summer days temperature remained higher from ca. 7 a.m. till 7 p.m. in the grassland than on the edge of shrubs and under the oak canopy, and differences in daily maxima often exceeded 8 °C. Mean photosynthetic photon flux density (PPFD) in the mid May – mid August period differed significantly among microhabitats. Grassland received full sun (2060 µmol photons m⁻² s⁻¹), Shrub plants 64% (1318 µmol photons m⁻² s⁻¹) and the oak subcanopy 3–4% (60–83 µmol photons m⁻² s⁻¹) of the direct solar radiation in the PAR spectrum. Due to a high spatial microheterogeneity in the shrub microhabitat Tree-Shrub plants experienced only half of the light (31% full sun, 660 µmol photons m⁻² s⁻¹) than Shrub plants. Volumetric soil water content decreased at all microsites from late April until early June, most sharply in the grassland from 22–26% to 7–10%, then increased again.

Table 1. Morphological attributes at shoot and canopy level for B. pinnatum growing in different microhabitats in situ in mid July 2000. Tree, Shrub and Grassland represent plants growing under the oak canopy, near the shrubs and in the grassland in situ, respectively. Significantly different (P < 0.05) microhabitat means in the same row are followed by different letters. The name of the parameter written in capital letters indicate that Kruskal-Wallis test and Dunn’s test were used to test for differences. In the other cases one-way ANOVA was used. For leaf number, internode length and stem thickness 3 replicates per treatment were calculated by averaging the values of 10 randomly chosen shoots. The last column presents the difference between the highest and the lowest microhabitat means expressed as percentage of the lowest mean ([(Max-Min)/Min] × 100, %).

* – Most shoots had only one node.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Microhabitat</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shrub</td>
<td>Grassland</td>
</tr>
<tr>
<td>Number of leaves per shoot</td>
<td>2.8b</td>
<td>2.1a</td>
</tr>
<tr>
<td>Internode length (cm)</td>
<td>7.2a</td>
<td>1.36b</td>
</tr>
<tr>
<td>Stem thickness (mm)</td>
<td>1.42b</td>
<td>1.36b</td>
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<tr>
<td>Leaf area index (LAI, m² m⁻²)</td>
<td>2.32a</td>
<td>1.74a</td>
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<tr>
<td>Standing crop (g m⁻²)</td>
<td>182.4b</td>
<td>123.2a</td>
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<tr>
<td>Leaf dry mass per unit soil surface area (g m⁻²)</td>
<td>107.4b</td>
<td>90.7b</td>
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<td>Stem dry mass per unit soil surface area (g m⁻²)</td>
<td>75b</td>
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<td>Specific leaf mass (SLM, g m⁻²)</td>
<td>47.7b</td>
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</tr>
<tr>
<td>Proportional leaf dry mass (%)</td>
<td>42b</td>
<td>26b</td>
</tr>
<tr>
<td>Proportional stem dry mass (%)</td>
<td>58a</td>
<td>74b</td>
</tr>
</tbody>
</table>

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in mid-summer. Soil was significantly drier (by 56–64%) for one or two treatments in the grassland than in the other two microhabitats in late May and early June. These differences in microclimate indicate that radiation load increases markedly in the direction of oak canopy < shrubs < grassland microhabitats, that may lead to higher frequency and/or longer periods of high temperature stress and temporal water shortage in summer, particularly in the grassland.

**B. pinnatum** canopy leaf area index (LAI) and aboveground standing crop

Leaf area index (LAI) for *B. pinnatum* was 48.7% and 11.5% higher near shrubs and in the grassland, respectively, than under the oak tree, although these differences were not statistically significant (Table 1) probably due to the relatively high variability of this parameter near shrubs. Furthermore, the unusually dry spring of
2000 may have substantially restricted leaf area development and thus obliterated between-habitat differences, particularly for the grassland. Indeed, average leaf number per shoot was significantly lower in the dry year 2000 than during the more precipitous 2001 (Table 1, Fig. 2b). Aboveground standing crop of B. pinnatum growing near shrubs significantly exceeded that under the oak tree (Table 1), where both leaf and stem dry mass per unit soil surface area were 2.5-times greater. Hence, the proportional dry matter invested into leaves and stems remained similar in these two microhabitats. In the grassland, standing crop was intermediate between that of Tree and Shrub microhabitats although the difference was not significant, and stem dry mass per unit shoot dry mass was markedly lower (Table 1). Stem dry mass per unit soil surface area in the grassland was similar to that under the oak tree, and was less than half of that near the shrubs. Leaf dry mass per unit soil surface area did not show significant differences from those of shrub and oak subcanopy microhabitats. A significantly lower specific leaf mass (SLM) was measured under the tree compared with the two other microsites. There was no significant difference in internode length between Tree and Shrub plants, but in the grassland most shoots had only one single node. Tree plants possessed significantly thinner stems than Grassland or Shrub plants (Table 1).

### Shoot morphology and leaf phenology

In each microhabitat, shoots of B. pinnatum emerged in early April and developed 2 – 3 leaves by the first date of data collection (20 April). In the growing season of 2001 shoots produced new leaves continuously until the end of July, every one or two weeks in May, but later at three or more weekly intervals. Total leaf area per shoot increased until about early July due to the increases in the number of leaves and – until early June – in the average size of individual fully developed leaves as well (except for grassland in situ, Fig. 2a–c). In September we observed newly emerged B. pinnatum shoots near shrubs and in the grassland (for transplanted plants as well), however these all died in winter.

Both the average size of fully expanded leaves and total leaf area per shoot of Grassland plants were greater than those of others in April (Fig. 2a, 2c), suggesting that B. pinnatum starts its phenological development earlier and/or faster in spring in the exposed grassland microhabitat than in the half- or full shade microenvironment. Maximum total leaf area of plants growing under the oak tree was about 35 – 40 cm², that is 25 – 45% lower than that of Grassland and Shrub plants or plants transplanted from the oak tree subcanopy to the other microhabitats (Fig. 2a). In the case of Shrub-Tree plants it was due to the lower area of single mature leaves, but in Tree plants fewer leaves also contributed to the small total leaf area per shoot (Fig. 2b–c). Note, that Shrub-Grassland plants produced leaves with hardly a greater area – both per leaf and per shoot – than those of the tree understory plants. For Tree and Shrub-Tree plants, however, the senescent part of total leaf area per shoot remained the lowest throughout the growing season (Fig. 2d). By mid August this was 20 – 30% under the tree, while in the grassland and for Tree-Shrub plants it amounted to about 54 – 60% being somewhat higher than even that of Shrub plants (49%). Also related to this, the estimated average leaf longevity was significantly higher for Tree plants than in the grassland or in the case of Tree-Shrub plants (Table 2).

### Chlorophyll a fluorescence induction

Maximum photochemical efficiency of PSII (Fv/Fm) in May was not much lower than the optimal value of 0.83 measured for most plant species (Maxwell & Johnson 2000), but it was significantly higher for Tree-Shrub plants and under the oak canopy than in the grassland or for Shrub plants (Fig. 3a). In June Fv/Fm tended to reduce in all plants, most strongly for Tree-Grassland plants (ca. 8%), although its value did not fall below 0.7. In April, before the canopy production of oaks and when the foliage of shrubs was not fully developed either,
Fig. 3a–e. Photochemical responses (chlorophyll fluorescence parameters) of transplanted and in situ grown B. pinnatum in different microhabitats in 2000. For abbreviations of treatments see Fig. 2. Statistically significant differences among treatment means of 3 (April) or 5 (May and June) replicates within the same month are indicated by small letters around symbols, and differences among months within the same treatment are indicated by capital letters below the name of the treatment. In both comparisons dissimilar letters indicate significant differences (P < 0.05). For three treatments, which photochemical responses were not measured in April, zeros are used instead of capital letters. a. Maximum photochemical efficiency of PSII (Fv/Fm) (dark-incubated chlorophyll fluorescence parameter), b. Effective quantum yield of PSII (ΦPSII), c. Photochemical fluorescence quenching (qP), d. Efficiency of PSII antennae (Fv'/Fm'), e. Non-photochemical fluorescence quenching (NPQ). For light-incubated fluorescence parameters (b–e) values recorded under the highest incubation photosynthetic photon flux density (1650 µmol photons m⁻² s⁻¹) are shown. The name of the treatment or the month written in capital letters indicates that Kruskal-Wallis test and Dunn’s test were used to test for differences among months within that treatment or among treatments within that month. In any other cases one- or two-way ANOVA were used.
there were not significant differences in effective quantum yield of PSII (ΦPSII) (and relative electron transport rate, ETR) and its components at 1650 μmol photons m⁻² s⁻¹ PPFD among leaves growing in the three microhabitats (with the exception of Shrub-Tree plants). This indicates that in all cases similar proportions of the light absorbed by chlorophyll associated with PSII were used in photochemistry (Fig. 3b–d). However, after the complete development of oak canopy, in May, newly flushed leaves of Tree and Shrub-Tree plants exhibited more than 70% lower photochemical quenching (qP, the proportion of excitons converted to electron transport) and ΦPSII accompanied with significantly higher efficiency of PSII antennae in capturing light and converting to excitons (Fv'/Fm’), and lower non-photochemical dissipation of excess light energy (NPQ) compared with leaves which were mature in April (Fig. 3b–e). As a consequence, in May qP became 4–7 times higher resulting in significantly higher ΦPSII, whereas Fv'/Fm’ tended to be 26–46% lower and NPQ about 1.5–2.5 times higher for leaves of Shrub plants and the plants of all three treatments growing in the grassland than for those under the oak canopy. Light response curves of ETR of Shrub plants and plants exposed to full sun in the grassland did not reach light saturation until high incident light (ca. 800–1000 μmol photons m⁻² s⁻¹, Fig. 4a). In April, leaves of Tree and Shrub-Tree plants showed light saturation pattern similar to those in the other two microhabitats, but ETR curves of leaves developed under the oak canopy in May became saturated at low light intensity (ca. 400 μmol photons m⁻² s⁻¹) resulting in threefold variation in ETR at 1650 μmol m⁻² s⁻¹ PPFD between the oak subcanopy and the two other microsites (Fig. 4a–b). Interestingly, Shrub-Tree plants exhibited even higher Fv'/Fm’ and lower NPQ than Tree plants (Fig. 3d–e). In Shrub plants qP and ΦPSII also diminished in May, but not so markedly (only about 20%) as in Tree and Shrub-Tree plants (Fig. 3c). Although Grassland plants differed significantly from Tree-Grassland, Shrub-Grassland and Shrub plants in qP (and also showed an opposite pattern between May and June), and in June Fv'/Fm’ as well, ΦPSII and NPQ were quite similar for these four treatments (Fig. 3b–e). ΦPSII (and ETR), qP and Fv'/Fm’ values obtained for Tree-Shrub leaves in May and June were intermediate between Tree and Shrub plants (Fig. 3b–d), but similar to Tree leaves; ETR reached a plateau at low PPFD (Fig. 4a). Only NPQ was as high as for Shrub plants (Fig. 3e). In June NPQ tended to increase in all plants, though this increase was the smallest in the grassland. For Shrub plants and the two transplant treatments in the grassland qP tended to decrease. It caused, however, a significant decline in ΦPSII only for Tree-Grassland plants (Fig. 3b–c). In contrast, ΦPSII changed hardly under the oak tree between May and June.

Leaf spectral reflectance

The analysis of leaf spectral reflectance showed that NDVI – estimating relative chlorophyll content – increased from May to June in both 2000 and 2001, except for the grassland microhabitat during the unusually dry spring of 2000, when NDVI declined, particularly for Tree-Grassland and Shrub-Grassland plants (Fig. 5a). REIP showed a similar, though less obvious trend (Fig. 5b). At a given sampling date significant differences between treatments appeared only in June 2000, when both NDVI and REIP were the lowest for plants transplanted from a shaded microenvironment to the exposed grassland (Tree-Grassland and Shrub-Grassland treatments), intermediate for plants near shrubs, and the highest for Tree and Shrub-Tree plants growing in a shaded microhabitat. In autumn, each of the three treatments in the grassland site produced new shoots where young leaves had markedly higher NDVI and REIP than leaves in June. In 2000 ΔPRI values – estimating VAZ xanthophyll cycle pool size – increased in all treatments from May to June (but only moderately in the grassland), than decreased again by October, in most cases below values recorded in May (Fig. 5c). Treatments differed more markedly in ΔPRI than in any other reflectance parameter measured (Table 3), and its value was 44–77% lower in May and 46–66% lower in June below the oak canopy than either near shrubs or in the grassland.

Relationship between leaf chlorophyll fluorescence and spectral reflectance

When the three microhabitats are compared there is negative correlation (r = −0.8858, P = 0.008) between ΔPRI and Fv'/Fm’, and positive correlation (r = 0.9434, P = 0.0014) between ΔPRI and NPQ: Leaves in oak shade possessed the lowest de-epoxidized xanthophyll cycle pigment content (lowest ΔPRI), the lowest NPQ and the highest PSII antennae efficiency (Fv'/Fm’). The opposite was true for treatments in the unshaded grassland (highest ΔPRI and NPQ, and lowest Fv'/Fm’), while plants near shrubs were either intermediate or similar to those in the grassland (Figs 5c, 3d–e). This correlation also held for seasonal changes, when e.g. the increase of ΔPRI from May to June was associated with an increase in NPQ and a decline in Fv'/Fm’ in most treatments (Figs 5c, 3d–e).

Leaf coarse structure

While specific leaf mass (SLM) differed only slightly in April, under the oak canopy it was about half of that near shrubs or in the grassland in May and June (Fig. 6a).
Table 3. Photochemical, spectral and structural leaf characteristics for *in situ* grown *B. pinnatum* in three different microhabitats in 2000. The upper and the lower values in the same cell were measured in May and in June respectively. For light-incubated fluorescence parameters values recorded under the highest incubation photosynthetic photon flux density (1650 mmol photons m\(^{-2}\) s\(^{-1}\)) are shown. For each variable 5 replicates were used. The last column presents the difference between the highest and the lowest microhabitat means expressed as percentage of the lowest mean \([(\text{Max}-\text{Min})/\text{Min}] \times 100\). (%).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Microhabitat</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shrub</td>
<td>Grassland</td>
</tr>
<tr>
<td><strong>Chlorophyll fluorescence</strong></td>
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<td></td>
</tr>
<tr>
<td>Maximum photochemical efficiency (Fv/Fm)</td>
<td>0.806</td>
<td>0.805</td>
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<tr>
<td></td>
<td>0.792</td>
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<td>Light-saturated electron transport rate (ETR)</td>
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<tr>
<td></td>
<td>53.9</td>
<td>54.7</td>
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<td>PSII quantum yield (ΦPSII)</td>
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<td>0.091</td>
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<td></td>
<td>0.078</td>
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<td>PSII antennae efficiency (Fv’/Fm’)</td>
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<td></td>
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<td>Photochemical quenching (qP)</td>
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<td></td>
<td>0.231</td>
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<td>Non-photochemical quenching (NPQ)</td>
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<td></td>
<td>4.48</td>
<td>4.30</td>
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<td><strong>Leaf spectral reflectance</strong></td>
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<td>Leaf normalized difference vegetation index (NDVI)</td>
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<tr>
<td></td>
<td>0.375</td>
<td>0.333</td>
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<td>Red edge inflection point (REIP, nm)</td>
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<td>705.3</td>
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<td>Difference in photochemical reflectance index (APRI)</td>
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<td>0.032</td>
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<td><strong>Leaf course structure</strong></td>
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<td>Thickness (mm)</td>
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<td></td>
<td>0.15</td>
<td>0.16</td>
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<tr>
<td>Bulk tissue density (g cm(^{-3}))</td>
<td>0.26</td>
<td>0.25</td>
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<tr>
<td></td>
<td>0.28</td>
<td>0.32</td>
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<tr>
<td>Specific leaf mass (SLM, g m(^{-2}))</td>
<td>39.6</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>41.7</td>
<td>50.9</td>
</tr>
</tbody>
</table>

![Fig. 4a–b.](image)

Mean values ± one SE are shown (in April n = 3, in May n = 5).

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Fig. 5. Leaf spectral reflectance parameters for transplanted and in situ grown B. pinnatum in different microenvironments.


For further explanation see text. For abbreviations of treatments see Fig. 2, and for representation of significant differences see Fig. 3. The name of the treatment or the month written in capital letters indicates that Kruskal-Wallis test and Dunn's test were used to test for differences among months within that treatment or among treatments within that month. In any other cases one-way ANOVA or unpaired t-test were used. For NDVI (a) and REIP (b) months or treatments without letter marks indicate that post tests were not significant among treatments within that month or among months within that treatment, respectively. For each variable 5 replicates were used.

Fig. 6a – c. Leaf structural characteristics for transplanted and in situ grown B. pinnatum in different microhabitats in 2000.

a. Specific leaf mass (leaf dry mass per unit leaf area, SLM, g m⁻²).
b. Leaf thickness (T, mm).
c. Leaf bulk tissue density (D = SLM/T, g cm⁻³).

For abbreviations of treatments see Fig. 2 and for representation of significant differences among means of 5 replicates see Fig. 3. The name of the treatment written in capital letters indicate that Kruskal-Wallis test and Dunn's test were used to test for differences among months within that treatment. In any other cases one- or two-way ANOVA were used.
Both components of SLM, i.e. leaf thickness and bulk tissue density were the lowest under the tree due to a significant decrease in leaf thickness of Tree and Shrub-Tree plants and to an increase in bulk leaf tissue density of the other plants in the grassland and near the shrubs in May (Fig. 6b–c). Shrub-Tree plants tended to have thinner leaves than Tree plants (Fig. 6b). In May SLM showed an intermediate value for Tree-Shrub plants, but its components did not differ significantly from those of Shrub plants (Fig. 6a–c). For Grassland plants leaves were also significantly thinner in May than in April (Fig. 6b). In May Tree-Grassland plants, in June Grassland and Shrub-Grassland plants produced leaves with significantly (ca. 21%) higher SLM than Shrub plants (Fig. 6a). This was mostly due to 17% thicker leaves of Tree-Grassland plants, but mostly to 15% and 18% higher leaf bulk tissue density of Grassland and Shrub-Grassland plants, respectively (Fig. 6b–c).

Discussion

Comparison of *B. pinnatum* plants growing in situ in contrasting microenvironments

As expected, leaf and shoot attributes for *B. pinnatum* differed most markedly between oak shade and grassland microenvironments representing the two extremes of the light regime. Significantly lower aboveground standing crop, total leaf area per shoot and PSII effective quantum yield in the oak understory suggest that light intensity in this microenvironment is limiting for the growth of *B. pinnatum*. Under such conditions light limitation may be ameliorated by increasing plant capacity for light capture at the expense of photosynthetic rate (e.g. Lambers et al. 1998; Givnish 1988). *B. pinnatum* showed this sort of adjustment both in shoot and leaf structure and in leaf photochemistry by producing shade type shoots under oak canopy and sun type shoots in the grassland and near shrubs. The opposite pattern of Fv’/Fm’ and qP in leaves growing in oak shade and in the other two, more exposed microsites (Fig. 3c–d) indicates differential chlorophyll partitioning. Leaves in oak shade appear to invest a greater amount of chlorophyll to light-harvesting complexes and PSII antennae (high Fv’/Fm’), whereas leaves in grassland or near shrubs appear to allocate relatively more chlorophyll into PSII reaction centers (high qP). During this adjustment variation in qP was remarkably high (5–7 times difference between oak shade and grassland) than in Fv’/Fm’ (53–71% variation, Table 3), as it was also found in other comparisons of sun and shade leaves (e.g. for *Ailanthus altissima*, Hamerlynck 2001 and *Fraxinus ornus*, Kalapos & Csontos 2003). Furthermore, *B. pinnatum* exhibited a greater difference in ΦPSII between leaves growing in the grassland and under the oak subcanopy (Table 3) than the 36% and 109% differences in this parameter reported for *A. altissima* (Hamerlynck 2001) and for *F. ornus* (Kalapos & Csontos 2003), respectively. Leaves in the grassland and shrub microenvironments showed higher NPQ than those in oak shade, although the variation was only moderate relative to that in qP (40–65% compared to 350–380%, Table 3). This variation was mainly due to a larger xanthophyll pigment pool size in the grassland and near shrubs than in oak shade (Figs. 3e, 5c). It is indicative of a higher capacity of leaves for photoprotection. Sun-grown plants are known to contain a larger total pool size of xanthophyll cycle components as well as a greater ability to convert this pool to zeaxanthin rapidly under high light (Demming-Adams & Adams 1996). Negative correlation between ∆PRI and Fv’/Fm’, and positive correlation between ∆PRI and NPQ confirm the greater importance of photoprotection by VAZ pigments in the grassland. Baker (1993) reported a high-light-induced decrease in Fv’/Fm’, indicative of a large increase in non-photochemical quenching for leaves of *Silene dioica* pretreated in high light. An increase in NPQ (and ∆PRI) from May to June experienced in our study may be associated with an increasing water shortage in this period (Fig. 3e). Veres et al. (1999) measured 100–120% increase in NPQ and also an increase of xanthophyll cycle pool pigments from early summer to the dry and warm July for several monocot and dicot species in a semiarid temperate sand grassland. For the congenereic *B. sylvaticum* Marchie & Horton (1998) also found relatively large changes in xanthophyll cycle pool size during shade acclimation.

Similarly to *B. pinnatum* in this study, many other single species comparisons reported sun leaves to have higher SLM than shade leaves (e.g. Abrams & Mostoller 1995; Naidu & DeLucia 1998; Kalapos & Csontos 2003), but the degree of variation can be different. The difference in SLM between full-shade- and full-sun-grown *B. pinnatum* leaves (Table 3) exceeds the 13.5% variation observed in this parameter between sun and shade leaves of *A. altissima* (Hamerlynck 2001) and is comparable to the 2–3-fold variation reported for *Fraxinus americana* and *Carya tomentosa* (Abrams & Mostoller 1995) as well as for *F. ornus* (Kalapos & Csontos 2003) growing in forest understory and open areas. Significant difference in SLM was also apparent in a greenhouse experiment with *B. pinnatum* between plants subjected to low and high light intensity treatments (de Kroon & Knops 1990b). In our study leaf thickness and leaf bulk tissue density appeared to play an equally important role for *B. pinnatum* in adjusting SLM to habitat light regime. This contrasts with the result of Ryser & Lambers (1995) for
B. pinnatum grown at different nutrient levels and with that of Garnier & Laurent (1994) for an interspecific comparison of annual and perennial pairs of grass species, who found leaf bulk tissue density to be the main determinant of SLM differences and thickness to have a smaller contribution. Greater amount of foliage on erect shoots requires relatively more investment in stems for support, as it is reflected in the higher stem thickness and higher dry mass per unit soil surface area for plants near shrubs than for those in oak shade. Grassland and Shrub plants had similarly thick stems, but the short stature of grassland plants resulted in stem dry mass per unit ground area as low as that in oak shade. Variation in LAI remained relatively moderate (49%) relative to that in aboveground standing crop and its components (145–157%, Table 1), that reflects the major contribution of coarse leaf structure to between-habitat differences. In the oak understory B. pinnatum showed a rapid and substantial plastic response to an altered habitat light regime when their sun type leaves in April under the leafless tree were followed by a shade type leaf generation in May after the overstorey foliage was completely developed (Fig. 4b). Continuous leaf production of B. pinnatum in spring and in early summer (Fig. 2b) may provide a greater opportunity for structural and biochemical adjustment to the seasonal light climate of the oak subcanopy microhabitat compared with that of some summegreen, forest understory species. Photosynthetic activity of these species also declines but in a smaller extent in response to the forest canopy closure through acclimation of leaves developed in early spring and still existing after the development of overstorey foliage (Fekete 1974; Rothstein & Zak 2001). High radiation load in the grassland microhabitat seems to have a beneficial effect for B. pinnatum in spring allowing higher photosynthetic activity and an earlier and/or faster start of plant phenological development probably through diminishing low temperature limitation on growth. However, the high degree of leaf senescence and low average leaf longevity in the grassland (Fig. 2d, Table 2) suggest that this xero-mesic grass suffers from water and high temperature stresses in the absence of at least partial shading by shrub or tree overstorey. The lack of a significant difference in leaf dry mass per unit soil surface area between these two microhabitats, despite the differences in specific leaf mass, is probably related to the significantly lower number of leaves in the grassland. High variation in the number of leaves observed in the same microhabitat between the two years or among treatments in 2001 (Table 1, Fig. 2b) does not agree with the result of De Kroon & Knops (1990a), who found that leaf number of this species did not differ substantially under different light conditions and nutrient availability in a greenhouse experiment. However, since leaf structure and photosynthetic electron transport rate of Shrub plants was similar to those in the grassland (Figs. 4a, 6a–c) it suggests that light limitation is markedly reduced for this species under half-shaded (shrub) microclimate relative to oak shade. At the same time, temporal water shortage seems to be less severe near shrubs than in the grassland as it is shown by a slightly smaller degree of leaf senescence near the shrubs than in the grassland (Fig. 2d).

Plastic responses of transplanted plants to their new microenvironment

After one year of transplantation, the morphology and photochemistry of transplanted B. pinnatum shoots differed markedly from those in their source microhabitat, and became highly similar to those growing in situ in their new microenvironment. Plastic responses in total leaf area per plant, SLM or PSII quantum yield seem to be most complete for plants transferred from half-shade (shrub) to shade (oak subcanopy) microhabitats (Figs. 2a, 6a, 3b). Even full-shade-grown B. pinnatum shoots were able to adjust to high light intensity after transfer from the oak subcanopy to the unshaded grassland, most clearly shown by the high ETR of their leaves (Fig. 4a). In May, ETR of these two (Shrub-Tree and Tree-Grassland) transfers differed about 3–4-fold from that in their source microhabitat (Shrub and Tree plants, respectively). Strauss-Debenedetti & Bazaz (1991) reported a similar degree of (2-6-fold) plasticity in maximum photosynthetic rates ($A_{max}$) of Cecropia obtusifolia and Ficus insipida, early successional tropical canopy emergents (Winter et al. 2001a, b), grown under low and high light conditions for 15 months, but only 40–50% difference for the late successional Pseudolmedia oxyphyllaria and Brosimum alicastrum. B. pinnatum showed small (not more than 6%) and insignificant differences in chlorophyll content expressed as either NDVI or REIP, but large differences in photochemical activity in May among plants growing in full-shade and half-shade or full-shade and full-sun microhabitats. This may indicate that adjustment at the chloroplast level – including changes in LHCII and/or Rubisco content – has a great importance in this species, contrasting with other grasses not found growing in shade at all and exhibiting photosynthetic acclimation predominantly by altering chlorophyll content at the leaf level, as well as with obligate shade species, like B. sylvaticum, which tends to show no such acclimation (Murchie & Horton 1997). By examining 22 herbaaceous species of various shade adaptation Murchie & Horton (1997) found the greatest flexibility at chloroplast level for plant species characteristic to habitats with spatially and/or temporally variable light environ-
Our results contrast with those of Loik & Holl (2001) from intraspecific comparisons of seedlings of four tree species grown under shrubs and in the grassland for 7 months. They did not find significant differences in photosynthetic capacity and apparent quantum yield for CO₂ uptake, despite the approximately 10-fold difference in the median PPFD between these two habitats; although these species all occur in the surrounding forest and do naturally colonize under shrubs and in open areas of abandoned pastures. However, the significant decrease in PSII quantum yield due to the decreasing tendency in the capacity for photochemical utilization of excitation energy (qP) during the hot and dry June of 2000 (Fig. 3b–c) indicates a possible photodamage on of excitation energy (qP) during the hot and dry June of 2000 (Fig. 3b–c) indicates a possible photodamage.

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In conclusion, our results confirm the hypothesis that B. pinnatum possesses rapid and substantial plasticity in leaf and shoot morphology, and in leaf photochemistry, that may contribute significantly to the species’ sustained persistence after deforestation. This allows even an expansion on microsites with different light regime in the course of secondary vegetation succession. Phenotypic plasticity is one of the most important mechanisms by which species respond to different microenvironmental conditions on the ecological time scale (Abrams & Mostoller 1995; Strauss-Debenedetti & Bazzaz 1991). Species native to spatially or temporally variable or unpredictable environments (e.g. early or mid successional plants) usually have flexible morphology and physiology, and can exceed in this capacity those from more homogeneous and stable environments (e.g. late successionals, Abrams & Mostoller 1995; Strauss-Debenedetti & Bazzaz 1991; Fekete 1974; Murchie & Horton 1997; Kalapos & Cson tos 2003). Being a dominant component of the understory and the edge of the former open xerothermic oak forests with heterogeneous light climate, B. pinnatum probably has evolved a high capacity for phenotypic plasticity. This is contrasted with the remarkably low light acclimation capacity of the congeneric obligate shade species B. sylvaticum (Murcie & Horton 1997). In addition, the clonal growth of B. pinnatum following the consolidation strategy may also promote its local persistence, and the non-plastic endogenous variation in rhizome length and bud activity may be of ecological importance in heterogeneous environments as well (de Kroon & Knops 1990a, de Kroon & Schieving 1990). However, it should be noted that the transplantation technique applied in our study was such that ramets became disconnected from their wider clonal connections. Even in this case leaves and shoots showed substantial acclimation capacity in morphology and photochemistry to the new microenvironment, that emphasizes the importance of flexibility of traits at the level of individual ramets in addition to the plasticity in clonal growth of B. pinnatum in its persistence in heterogeneous environments. As transplanted plants revealed, adjustment of B. pinnatum was more complete in the local environment of shaded microhabitats, where low light intensity is the main limiting factor, than in the full sun microenvironment of the grassland, where high light intensity is associated with high temperature and water stresses. These components of high radiation load limited plant assimilation and even caused moderate damage to leaves in a drier-than-average year. This may be related to the long-term gradual decline in abundance and vitality of this species through secondary succession in the absence of tree or shrub shading. Our results confirmed that B. pinnatum attains its best growth in the half shade of shrubs, where both light limitation and water stress are less severe. This observation further supports the assumption that the forest edge or open woodland phytocoenological affinity of B. pinnatum in Central Europe (Ellenberg 1988) can be explained – at least partly – by the species’ ecophysiological tolerance.

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