Community Seasonal Development Enables Late Defoliation Without Loss of Forage Quality in Semi-natural Grasslands

Jan Mládek · Michal Hejcman · Stanislav Hejduk · Martin Duchoslav · Vilém Pavlů

Abstract Agri-environmental measures often require postponing of grassland defoliation until summer months. We investigated how this affects agronomic characteristics, i.e., biomass production and forage quality, in species-rich grasslands in the White Carpathian Mountains, Czech Republic. Three distinct grasslands (vegetation alliances *Bromion erecti*, *Cynosurion cristati*, *Violion caninae*) were selected for biomass sampling in three dates: mid-May, early June and end of June. Proportions of individual species from total biomass, biomass production, crude fibre concentration, organic matter digestibility and community functional properties (forage value, leaf dry matter content, specific leaf area) were determined. Dry matter standing biomass at the end of June was highest in *Bromion* with 3.5 t/ha, followed by *Violion* with 2.7 t/ha and *Cynosurion* with 2.3 t/ha. A steep decline in forage quality (increase in crude fibre and decrease in organic matter digestibility) during accumulation of above-ground biomass was recorded in formerly unmanaged *Bromion* grassland but remarkably not in formerly grazed *Cynosurion* and *Violion* grasslands where early spring dominants were partly replaced by later developing species, *Agrostis capillaris* and *Trifolium* spp. Abundance-weighted community functional properties were

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consistent with results obtained from biomass chemical analyses, thus the 'trait approach' can be used as a suitable surrogate of costly and labor-intensive laboratory procedures. Finally, we assume that the high degree of community seasonal development in *Cynosurion* and *Violion*, indicated here by a new community seasonal development index and by development in community specific leaf area, was accountable for the stabilization of forage quality later in the vegetation season.

Keywords Biomass production · Community functional properties · Community seasonal development index · Legumes · Organic matter digestibility · Species-rich grasslands

Abbreviations
CSDI   Community seasonal development index
LDMC   Leaf dry matter content
OMD   Organic matter digestibility
SLA   Specific leaf area

Plant nomenclature Kubát et al. (2002)

Introduction

Vast areas of species-rich grasslands throughout Europe are incorporated into agri-environmental schemes that compensate farmers for any loss associated with measures that aim to benefit the environment or biodiversity (Kleijn and Sutherland 2003). Grassland extensification schemes usually postpone defoliation activities to June–July to assure diaspore production of endangered plant species and reproduction of insects and ground nesting birds (Albrecht et al. 2007). One question that has not yet received sufficient study is, how does postponed defoliation affect biomass production and forage quality in different vegetation types and, therefore, profitability of livestock production systems.

For a long time, agronomists have focused on intensive grassland management, especially on agronomic characteristics (biomass production and forage quality) in productive sown grasslands (Frame 1994), whereas ecologists have tackled the issues of vanishing biodiversity (Bakker 1989). For these reasons, research of agronomic characteristics of semi-natural species-rich grasslands has received little attention, although such information is vital for the planning of grazing or cutting management in agri-environmental measures (Bokdam and Wallis De Vries 1992). Biomass production of particular vegetation units has been seldom investigated (Oomes and Mooi 1981; Schino et al. 2003; Hrevušová et al. 2009; Heinsoo et al. 2010), and forage quality in semi-natural grasslands obtained from late harvesting has also received little attention (Bruinenberg et al. 2003; Fiems et al. 2004; Bovolenta et al. 2008; Hessle et al. 2008; Čop et al. 2009). The studies that were conducted generally concluded that forage quality was low and only suitable for low-performing animals.

Regarding the seasonal development of forage quality in intensive grasslands, crude fibre concentration increases, mean nitrogen and phosphorus concentrations in forage dry matter decrease with increasing standing biomass (dilution effect), with decreasing

leaf:stem ratio and biomass senescence (Duru and Ducrocq 1997; Duru et al. 2008; Gibson 2009). Therefore, organic matter digestibility (OMD) during season usually quickly decreases and livestock performance is notably reduced (Frame 1994). However, semi-natural species-rich grasslands may host a great variety of dicotyledons that differ from the main forage grasses by, in most cases, having late-seasonal growth (Mitchley 1988; Martínková et al. 2002) and by higher OMD of stems, and particularly in leaves maintaining almost constant OMD over the growing season (Duru 1997). In spring, the proportion of leaves in the aboveground dry matter decreases faster in nutrient-rich than in nutrient-poor plots (Duru and Ducrocq 1997) or plant communities (Calvière and Duru 1999). A faster decline of OMD in highly productive compared with low productive plant communities may be therefore expected.

Several recent studies (e.g., Garnier et al. 2004; Ansquer et al. 2009; Duru et al. 2009; Fortunel et al. 2009) provided evidence that grassland properties (including primary productivity, OMD) are well correlated with plant functional traits. Indeed, OMD for a wide range of grass species from semi-natural grasslands correlates negatively with leaf dry matter content (LDMC) and positively with specific leaf area (SLA) (Al Haj Khaled et al. 2006; Pontes et al. 2007).

The defoliation regime is a key factor determining phenological patterns in a grassland community (Mitchley 1988; Smith and Jones 1991; Bergfur et al. 2004). In productive fallow stands and summer mown meadows, the main factor driving species growth is competition for light; consequently dominant species are synchronous in their development (Martínková et al. 2002). However, productive stands exposed to repeated defoliation permit the coexistence of slower developing species with dominants and thus support high functional diversity (sensu plant height, flowering time, LDMC) in plant communities (Al Mufti et al. 1977; Adler et al. 2001; Questad and Bryan 2008). Functionally diverse communities show less variation of standing biomass around the peak (Ansquer et al. 2009), and enable extending the cutting/grazing dates without a large effect on the amount of harvested forage. However, no studies have tested which plant communities enable postponing defoliation until summer months without a significant decline in forage quality.

Several metrics appropriate for the evaluation of community temporal changes have recently been recommended (e.g., Frost et al. 1995; Collins et al. 2008), but all these metrics are designed for the description of temporal changes in permanent plots and are not suitable for destructively sampled biomass data. Therefore, we introduced a new measure of community seasonal development which appears from "Canberra Metric Similarity Measure" by Lance and Williams (1967).

In this paper, grasslands in the White Carpathian Mountains were investigated because of their extraordinary species-richness, up to 103 vascular plant species per 24 m^2 (Jongepierová et al. 2007), which has been predetermined by a long history of low input management and by the biogeographical position situated between the Subatlantic, Montane Carpathian and Pannonian regions. Three types of the most widespread grassland communities in the region (Škodová et al. 2008) were selected for detailed analysis: *Bromion erecti* Koch 1926 (henceforth *Bromion*), *Cynosurion cristati* Tüxen 1947 (*Cynosurion*) and *Violion caninae* Schwickerath 1944 (*Violion*), i.e., three distinct vegetation alliances as described in Chytrý (2007). These grasslands possess high conservation values in central Europe; moreover, *Bromion* and *Violion* are included in the list of Natura 2000 priority habitats (Chytrý et al. 2001).

The aim of this study was to answer the following questions: *i*) Do selected grasslands differ in biomass production? *ii*) How is biomass production and forage quality affected by postponed defoliation? *iii*) Do selected grasslands differ in seasonal development of plant species composition? *iv*) Is it possible to explain seasonal development of forage quality determined by chemical analyses of biomass by means of seasonal change in community forage value, community LDMC and community SLA in selected grasslands?

Material and Methods

Study Sites

The study was carried out in the White Carpathians Mountains situated in the borderland between the Czech Republic and Slovakia. The soils in all sites are haplic cambisols, developed on tertiary flysch sediments that consist of alternating sandstone and rock clay layers of variable thickness. Three sites were selected where distinct communities have developed under different management histories on soils with highly contrasting properties (see Table 1): 1) *Bromion* (49°05′58″ N, 18°01′59″ E) had been unmanaged for 13 years prior to the start of the study, formerly managed by cattle grazing, 2) *Cynosurion* (48°56′20″ N, 17°48′00″ E) had been rotationally grazed by cattle from early spring for more than 25 years prior to the start of the study, 3) *Violion* (48°53′47″ N, 17°34′44″ E) was old, eroded and continuously grazed pasture on a hilltop with a history of more than 50 years of sheep grazing beginning each year in early spring.

Table 1 Description of investigated sites

Site		*Bromion*	*Cynosurion*	*Violion*
Altitude	m a.s.l.	370	720	450
MAT	°C	7.9	6.0	8.0
MAP	mm	760	850	669
Species richness	per 1 m^2	32	33	30
Clay	%	28	10	9
Silt	%	66	69	35
Sand	%	6	23	56
pH (CaCl$_2$)	Units	5.4	5.0	4.6
P	mg/kg	7.4	7.8	9.0
K	mg/kg	342	245	99
Ca	mg/kg	3,539	1,963	1,102
Mg	mg/kg	218	204	67

MAT – mean annual temperature, *MAP* – mean annual precipitation; plant-available contents of P, K, Ca and Mg in soil determined with Mehlich III.

Sampling Design, Biomass Production and Forage Quality Analyses

In 2004, 15 permanent 5 m × 5 m plots were established in each investigated grasslands (45 permanent plots in all three sites together). In each 5 m × 5 m plot, three 1 m × 1 m sub-plots were randomly selected for data collection on three sampling dates, i.e., one sub-plot was cut in each sampling date only (Fig. 1). Such a systematic sampling design enabled the bias in temporal development of biomass characteristics caused by spatial heterogeneity in species composition to be minimized. All plots were fenced off before data collection to prevent them from any unwanted defoliation. Because of the complex analyses of agronomic characteristics and plant species composition, selection of more sites with the same plant community was not feasible. Furthermore, only two sampling dates in *Cynosurion* and *Violion* were used because of technical problems.

Sampling dates were 19th May, 7th June, and 30th June. Cutting in mid-May has been recommended as the best time for hay production on two cut meadows in central Europe because it offers the best compromise between forage yield and its quality (Buchgraber and Gindl 2004). The 30th June, however, was recognized as the first possible day of harvest enabling generative reproduction of endangered plant species in the investigated grasslands (Háková et al. 2004). Sub-plots had been neither grazed nor cut since start of the vegetation season, and thus they always provided first-growth biomass in all sampling dates. Biomass from each sub-plot was harvested 3 cm above ground, weighed and taken to the laboratory and dried at 55°C until total desiccation was reached. Dry matter content was determined and dry matter standing biomass was calculated for each sub-plot.

Weende analysis was used to determine crude fibre (AOAC 1984) and in vitro OMD was analyzed according to Tilley and Terry (1963). Both parameters of forage quality were analyzed from each biomass sample.

Fig. 1 Scheme of biomass sampling in grasslands, three subplots within each 5 m × 5 m permanent plot present sampling dates 1) 19th May, 2) 7th June, 3) 30th June. Arrangement of plots is not strictly contiguous because these established plots were, after this baseline data collection, used for management experiment with grazing plots at the edges
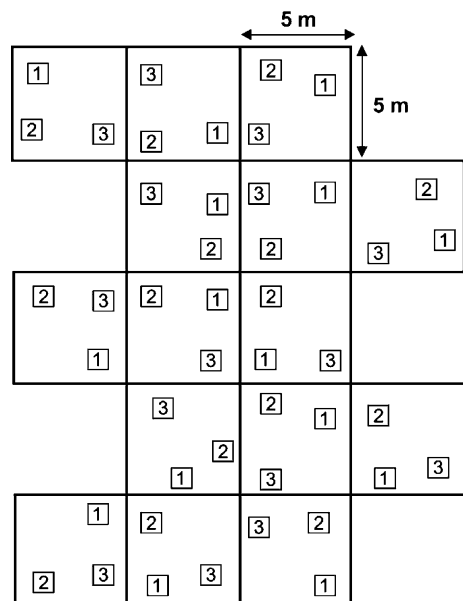
Plant Species Composition and Community Seasonal Development

Species biomass in a sample was estimated using the calibrated weight-estimate method (Tadmor et al. 1975). In several training plots visual estimates of species biomass were calibrated by clipping and weighing, and when consistent estimates were attained, estimations of species biomass in studied sub-plots were undertaken. Further, proportion of each species from the total sample biomass was calculated. The sum of proportions of all species present within the sub-plot was 100%. As seasonal development of community plant species composition was evaluated from destructive biomass sampling undertaken in each term in the new sub-plots, proportions of species from all 15 sub-plots were averaged and species ranked in declining order. Subsequently, species whose cumulated biomass reached 80% of community standing biomass (taken as an average from two or three sampling terms) were analyzed for temporal development; in our case 14 of the most abundant species were taken in all three grasslands. Analysing species accounting for 80% of the total biomass has been recognized to be sufficient for the capture of community properties (Garnier et al. 2004; Fortunel et al. 2009).

For quantification of community temporal turnover, the community seasonal development index (CSDI) was proposed (Eq. 1). It is derived from the "Canberra Metric Similarity Measure" (Lance and Williams 1967), which so far has been used by ecologists for the evaluation of species abundance similarity between two samples. Our modification quantifies a temporal change of the proportions of the most abundant species in a community.

$$CSDI_n = \frac{1}{n} \sum_{i=1}^{n} \frac{|x_i - y_i|}{(x_i + y_i)} \tag{1}$$

where x_i is an average proportion of species i from all sub-plots at the locality on the initial (spring) sampling date and y_i is an average proportion of species i from all sub-plots at the locality on subsequent (summer) sampling date and n is the number of the most abundant species with a cumulated biomass proportion reaching approximately 80% (taken as an average from both sampling terms).

The index has a range of 0–1 and following desirable characteristics: the minimum value 0 is applied if there has been no change from initial (spring) proportions of all species, and the maximum value 1 is only reached when all initial (spring) species have been completely replaced by other species appearing first in the subsequent (summer) sampling term. The index is symmetrical (interchanging x_i and y_i gives same result) but nonlinear, i.e., a unit change in $|x-y|$ does not have the same effect at all values of x. This means that it is sensitive to proportional rather than absolute differences, for example, CSDI gives a value ten times higher to species change from a proportion of 0.01 to 0.1 than from a proportion of 0.51 to 0.60. This seems to us to be a biologically sensible transformation, since later-developing species (e.g., legumes) bring young digestible tissues into the community biomass. Although minor, these tissues often synergistically improve the OMD of the community biomass as a whole due to associative effects of high nitrogen concentration plants on the digestion process of biomass with a poor forage quality (Niderkorn and Baumont 2009). Conversely, initial dominants increase their proportions mainly with structural

material, which is hardly digestible, therefore dominant change (is often higher in absolute values) is deliberately not emphasized. The index is vulnerable to sampling errors for rare species, therefore only the most abundant species with cumulated biomass proportions reaching 80% should be taken into consideration.

For a clear visualization of the index characteristics, contours of the CSDI were plotted for all combinations of x (spring proportion, range 0–1) and y (summer proportion, range 0–1) in the case of two species in a sample (Fig. 2). Since CSDI had to be expressed as a function of two variables for graph construction, Eq. 1, for two species:

$$CSDI_2 = (|x_1 - y_1|/(x_1 + y_1) + |x_2 - y_2|/(x_2 + y_2))/2 \qquad (2)$$

was modified by substituting the proportions of the second species from their definition by

$$x_2 = 1 - x_1 \qquad (3)$$

and

$$y_2 = 1 - y_1 \qquad (4)$$

After substitution, Eq. 2 for graph construction may be rewritten as follows:

$$CSDI_2 = (|x_1 - y_1|/(x_1 + y_1) + |x_1 - y_1|/(2 - x_1 - y_1))/2 \qquad (5)$$

Community Functional Properties

Based on the assumption of the "biomass ratio hypothesis" (Grime 1998), functional parameters at the community level were calculated by weighing the trait value of

Fig. 2 The values of a community seasonal development index (CSDI) as a function of spring (x) and summer (y) proportions when two species are available
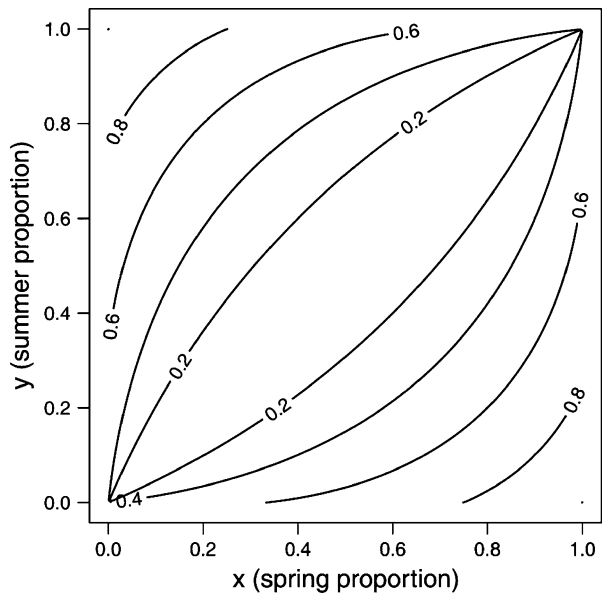
species with their relative contributions to the sample biomass (Fortunel et al. 2009). The community forage value, community LDMC and community SLA were calculated for each sub-plot.

To calculate the community forage value, forage indicator values of individual species were taken from the BIOLFLOR database (Klotz et al. 2002). Forage indicator values ranged from 1 (lowest) to 9 (highest). Out of a total of 89 recorded species, nine lacked forage indicator values in the BIOLFLOR database which is why missing values were supplemented from the database by Jurko (1990) and adapted to the BIOLFLOR scale. The tabular LDMC (average of aggregated records) and tabular SLA (average of aggregated records) of individual species were taken from the LEDA trait-base (Kleyer et al. 2008). Community LDMC and community SLA were calculated using 78 and 85 species, respectively, as data for 11 and four species (fortunately rare in samples) were not available.

Data Analysis

Plotting values of CSDI in a model example of two species in a sample was performed with R 2.10.1 software (www.r-project.org). Further analyses were performed using the statistical package SYSTAT 13 (www.systat.com). Prior analyses data were checked for normality and homogeneity of variances and when necessary they were log-transformed. In the statistical analyses of standing biomass, crude fibre, OMD and community functional properties (response variables), site (*Bromion*, *Cynosurion*, *Violion*) and date (19th May, 7th June, 30th June) were in ANOVA considered as fixed factors (explanatory variables). Technical problems caused that there were no observations for some cells in a standard two-way ANOVA, such data were difficult to analyze. Therefore, simpler means model was used that treats the analysis as a large single factor ANOVA comparing all cells with tests for main effects and interactions. Contrasts based on cell means were used for the tests of sensible hypotheses according to recommendations of Quinn and Keough (2002: 244–247). The null hypotheses were as follows: Site: *Bromion vs Violion* for the first and the last sampling date ($\mu_{Bromion\_19\ May}+\mu_{Bromion\_30\ June}=\mu_{Violion\_19\ May}+\mu_{Violion\_30\ June}$), *Bromion vs Cynosurion* for the second and the last sampling date ($\mu_{Bromion\_7\ June}+\mu_{Bromion\_30\ June}=\mu_{Cynosurion\_7\ June}+\mu_{Cynosurion\_30\ June}$), and *Cynosurion vs Violion* for the last sampling date ($\mu_{Cynosurion\_30\ June}=\mu_{Violion\_30\ June}$); Date: the first *vs* the last date for *Bromion* and *Violion* ($\mu_{Bromion\_19\ May}+\mu_{Violion\_19\ May}=\mu_{Bromion\_30\ June}+\mu_{Violion\_30\ June}$), the second *vs* the last date for *Bromion* and *Cynosurion* ($\mu_{Bromion\_7\ June}+\mu_{Cynosurion\_7\ June}=\mu_{Bromion\_30\ June}+\mu_{Cynosurion\_30\ June}$); Site×date: *Bromion vs Violion* at the first *vs* the last date ($\mu_{Bromion\_19\ May}-\mu_{Violion\_19\ May}-\mu_{Bromion\_30\ June}+\mu_{Violion\_30\ June}=0$), *Bromion vs Cynosurion* at the second *vs* the last date ($\mu_{Bromion\_7\ June}-\mu_{Cynosurion\_7\ June}-\mu_{Bromion\_30\ June}+\mu_{Cynosurion\_30\ June}=0$). Post hoc comparisons on the least squares means were conducted using Fisher's LSD as recommended by Milliken and Johnson (1984). Further, seasonal development of each of 14 the most abundant species within each site was compared by one-way ANOVA and Tukey's HSD post-hoc test was applied to evaluate differences between sampling dates after obtaining significant ANOVA result.

Results

Biomass Production and Forage Quality

The amount of dry matter standing biomass ranged from 0.8 to 3.2 t/ha over all of the sites and sampling dates (Fig. 3a). The standing biomass was greater in *Bromion*
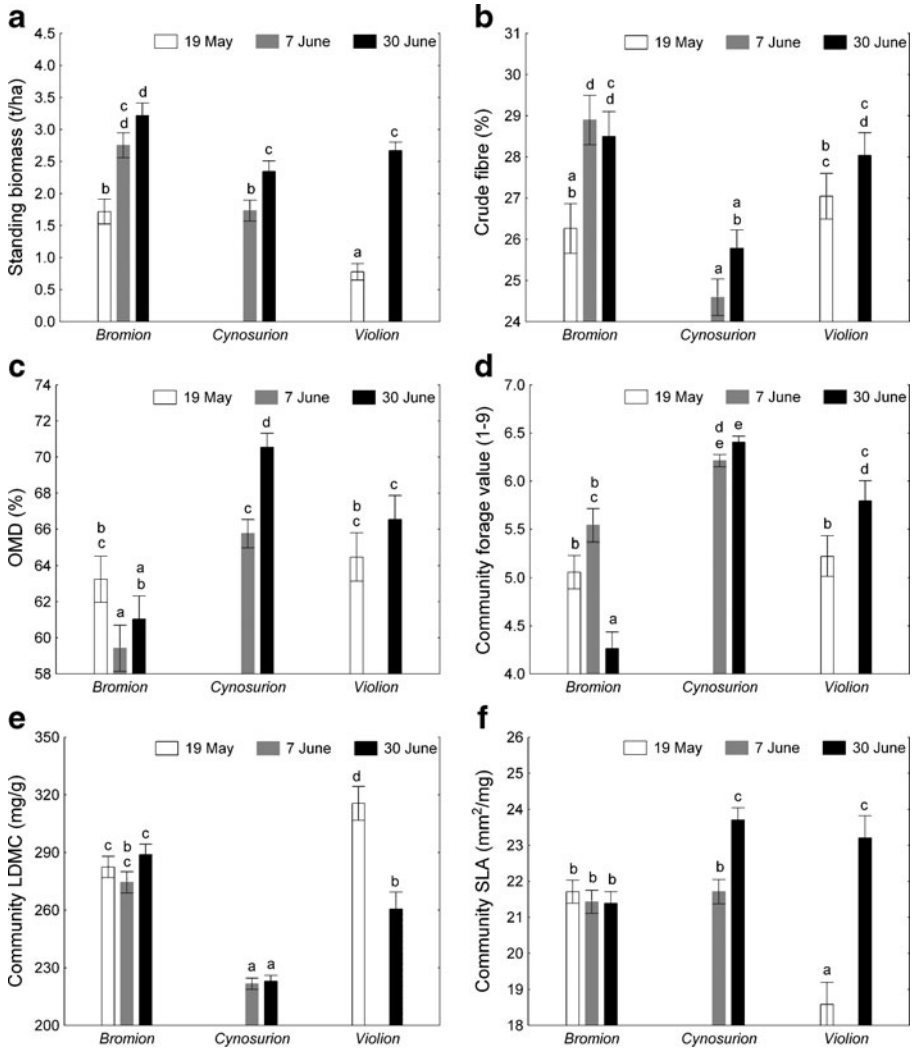

Fig. 3 Seasonal development of (**a**) dry matter standing biomass, (**b**) crude fibre concentration, (**c**) *in vitro* organic matter digestibility (OMD), (**d**) community forage value, (**e**) community leaf dry matter content (LDMC) and (**f**) community specific leaf area (SLA); error bars represent SE; means with the same letter are not significantly different (Fisher's LSD test, $P<0.05$)

than in other sites throughout the season (site: $F=43.22$, $P<0.001$) and increased during the season at all sites except for *Bromion* where it stagnated in the last two sampling dates (date: $F=45.56$; site×date: $F=9.69$, all $P<0.001$).

Regarding forage quality, crude fibre concentrations ranged from 24.6% to 28.9% over all of the sites and sampling dates (Fig. 3b). It significantly differed among sites ($F=13.33$, $P<0.001$) and dates ($F=3.83$, $P=0.05$). There was also significant effect of site×date interaction ($F=10.50$, $P<0.001$). *Cynosurion* had significantly lower crude fibre concentration than other sites. During the season, a significant increase in crude fibre concentration was recorded in *Bromion* but no significant changes were observed in other sites. OMD ranged from 59.4% to 70.5% over all of the sites and sampling dates (Fig. 3c). It significantly increased in the direction *Bromion* → *Violion* → *Cynosurion* ($F=15.96$, $P<0.001$) but except for its decrease from the first to the second sampling date in *Bromion* and increase from the second to the third sampling date in *Cynosurion*, no changes over season were observed (date: $F=2.99$, $P=0.057$; site×date: $F=10.50$, $P<0.001$).

Community Functional Properties

Community forage value ranged from 4.3 to 6.4 over all of the sites and sampling dates (Fig. 3d); it was the highest in *Cynosurion*, followed by *Violion* and the lowest in *Bromion* (site: $F=28.78$, $P<0.001$). Seasonal development of community forage value was site-specific (date: $F=4.43$, $P<0.001$; site×date: $F=12.29$, $P<0.001$). While significantly decreased in the last sampling date in *Bromion*, it was stable in *Cynosurion* and increased in *Violion*. Community LDMC (Fig. 3e) was stable in *Bromion* (high values) and *Cynosurion* (low values) while strongly changed in *Violion* from high to middle values over season (site: $F=28.21$, $P<0.001$; date: $F=6.54$, $P=0.005$; site×date: $F=10.97$, $P<0.001$). Community SLA (Fig. 3f) was stable in *Bromion* (middle values) while strongly changed from low or middle to high values over season in *Violion* and *Cynosurion*, respectively (site: $F=2.94$, $P=0.037$; date: $F=11.05$, $P<0.001$; site×date: $F=16.17$, $P<0.001$).

Plant Species Composition

In *Bromion*, four out of 14 of the most abundant species exhibited significant seasonal development. The proportion of the dominant *Brachypodium pinnatum* had increased substantially while proportions of *Poa pratensis* and *Dactylis glomerata* had significantly decreased by the last sampling date (Fig. 4a). In *Cynosurion*, two out of 14 of the most abundant species exhibited significant seasonal development. The proportion of *Agrostis capillaris* had substantially increased whilst that of *Knautia kitaibelii* had significantly decreased (Fig. 4b). In *Violion*, seven out of 14 of the most abundant species exhibited significant seasonal development (Fig. 4c). Proportions of *Festuca rubra*, *Agrostis capillaris*, *Trifolium repens* and *T. pratense* had significantly increased while proportions of *Bromus erectus*, *Festuca rupicola* and *Poa pratensis* had significantly decreased by the last sampling date in comparison to the first sampling date.

In *Bromion*, CSDI calculated with biomass proportions of 14 of the most abundant species was 0.30 for community change from 19th May to 7th June and 0.39 for

change from 7th June to 30th June, and 0.39 was also reached for change from 19th May to 30th June. In *Cynosurion*, CSDI was 0.38 for community change from 7th June to 30th June and in *Violion*, CSDI was 0.54 for community change from 19th May to 30th June.

Discussion

Biomass Production

As expected from soil properties of the sites (Table 1, *Bromion* had substantially higher pH, K, Ca and Mg contents than other two sites), dry matter standing biomass was the highest in *Bromion* and the peak standing biomass of 3.2 t/ha was comparable to the maximum levels measured in *Bromion erecti* grasslands throughout Europe (Ryser et al. 1995; Schino et al. 2003; Smits et al. 2008). Grasslands dominated by *Brachypodium pinnatum* or *Bromus erectus*, common grasses exhibiting resource conservation strategies (Duru et al. 2009), seem to have maximum standing biomass at around 4 t/ha. In *Violion*, the peak standing biomass of 2.7 t/ha was consistent with amounts measured for various *Nardus* grasslands similar to *Violion caninae* (Bakker et al. 2002; Schino et al. 2003; Hejcman et al. 2006, 2007). The maximal standing biomass of grasslands with *Nardus stricta* seems to be around 3 t/ha. In *Violion caninae* and *Bromion erecti* grasslands, the increase in biomass production above maximal limits is connected with the expansion of highly productive species from different alliances and therefore occurs with changes in grassland communities. This is clearly visible from long-term fertilizer experiments where an increase in nutrient availability eliminated species typical for *Violion caninae* in favour of more productive species typical for *Arrhenatherion elatioris* alliance (Hejcman et al. 2010a).

Although *Cynosurion* possessed intermediate soil properties, the standing biomass here was not different from *Violion* on 30th June. The recorded standing biomass of 2.3 t/ha contrasted with that of 5–7 t/ha reported for *Cynosurion cristati* grasslands by other studies (Pavlů et al. 2003; Schino et al. 2003; Hejcman et al. 2010b). Lower standing biomass found in *Cynosurion* can be partly explained by the delayed growth due to higher altitude and by the adaptability of the sward to frequent defoliation which supports short species able to easily regenerate after defoliation. A maximal yield can probably be obtained in this grassland by frequent defoliation, not by the postponing of the first defoliation to the end of June.

Furthermore, in contrast to *Violion caninae* and *Bromion erecti* communities with clear upper biomass production limits, *Cynosurion cristati* grasslands can be highly variable in standing biomass as its characteristic species are adapted to frequent defoliation under a wide range of nutrient availabilities (Jurko 1969). This is the reason why the upper biomass production limit in *Cynosurion cristati* grasslands can hardly be estimated.

Forage Quality

The seasonal patterns of forage quality development in the selected sites differed greatly. From mid-May to the end of June, tall sward *Bromion* exhibited a slight

Fig. 4 Seasonal development of 14 of the most abundant species in (a) *Bromion*, (b) *Cynosurion* and (c) ▶
Violion; error bars represent SE; n.s. – not significant result of one-way ANOVA; * – $P<0.05$; ** – $P<$
0.01; *** – $P<0.001$; sampling dates within each species with the same letter in *Bromion* are not
significantly different (Tukey's HSD test, $P<0.05$); number above bar for individual species is its forage
indicator value (according to BIOLFLOR database; see Methods) Species abbreviations: AchMil –
Achillea millefolium, AgrCap – *Agrostis capillaris*, AgrEup – *Agrimonia eupatoria*, AlcSpe – *Alchemilla*
sp., AstDan – *Astragalus danicus*, BraPin – *Brachypodium pinnatum*, BroEre – *Bromus erectus*, CenJac –
Centaurea jacea, CynCri – *Cynosurus cristatus*, DacGlo – *Dactylis glomerata*, FesPra – *Festuca
pratensis*, FesRub – *Festuca rubra*, FesRup – *Festuca rupicola*, FraVir – *Fragaria viridis*, GalAlb –
Galium album, GalVer – *Galium verum*, HerSph – *Heracleum sphondylium*, KnaKit – *Knautia kitaibelii*,
LatPra – *Lathyrus pratensis*, LeoHis – *Leontodon hispidus*, MedFal – *Medicago falcata*, NarStr – *Nardus
stricta*, PimSax – *Pimpinella saxifraga*, PoaPra – *Poa pratensis*, PotRep – *Potentilla reptans*, TriFla –
Trisetum flavescens, TriMed – *Trifolium medium*, TriPra – *Trifolium pratense*, TriRep – *Trifolium repens*

decline in OMD and a significant increase in crude fibre concentration (and
significant declines of N, P and K biomass concentrations, unpubl. data), whereas
the short sward communities *Cynosurion* and *Violion* displayed an unexpected
increase or stability of OMD and stability of crude fibre concentration (the same
pattern was detected for N, P, K, Ca and Mg biomass concentrations, unpubl. data).
Such patterns might be attributed to the higher nutrient supply (indicated by soil
properties, Table 1) in *Bromion* producing a "dilution effect" (Duru and Ducrocq
1997) through growth of long stems with lower nutrient concentrations and lower
OMD than leaves (Calvière and Duru 1999). Furthermore, an increase in
dominance of *Brachypodium pinnatum*, with a high proportion of senescent
leaves, was recorded in *Bromion* in the last sampling date. Stable forage quality in
Cynosurion and *Violion* contrasted with frequently reported declines in forage
quality in the case of postponed defoliation in species-poor intensive grasslands
(Bruinenberg et al. 2002). *Cynosurion* and *Violion* could manifest such forage
quality patterns due to their long grazing histories with early spring defoliation,
which enabled the coexistence of slower developing species and also supported
more digestible species occurring often in frequently defoliated sites (Louault et al.
2005; Čop et al. 2009). Differences in OMD between low altitude *Bromion* and
upland *Cynosurion* could be partly because the lignification of cell wall material
increases at high temperatures and accumulation of digestible storage products is
greater at low temperatures (Gibson 2009). Therefore, on the basis of our results it
is not possible to declare that the described seasonal patterns of forage quality are
typical for these vegetation units in general. But Hejcman et al. (2010c) recently
reported from a long-term fertilization experiment that late cutting management
decreases forage quality more in highly productive (*Arrhenatherion elatioris*) than
in low productive (*Violion caninae*) plant communities and this is consistent with
our results.

The OMD ranged from 59% to 71% and, as we expected, was lower than for the
standard forage grass, *Lolium perenne* with an average digestibility of 80%
(Bruinenberg et al. 2002). It can be concluded that the biomass from all of the
investigated sites was of low quality and could not be used for highly intensive milk
production systems. Instead, the biomass from the investigated grasslands could
satisfy the lower nutritional requirements of low-performance beef cattle or sheep.
This conclusion corresponds to the results by Bokdam and Wallis De Vries (1992)
from semi-natural grasslands in the Netherlands.
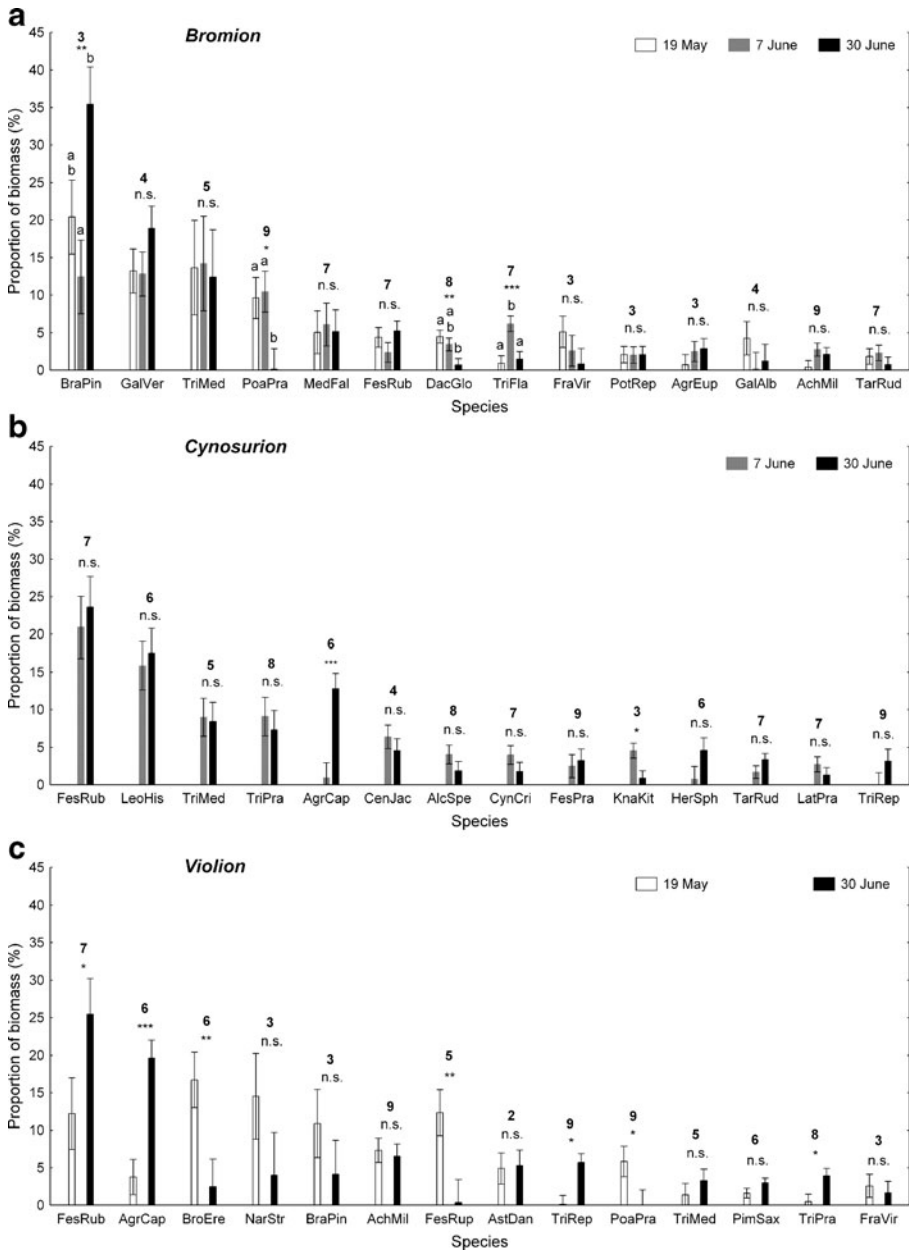
Plant Species Composition and Community Functional Properties

The development of community functional properties, derived from species composition, was well reflected in the divergent seasonal development of biomass quality parameters (crude fibre, OMD) in *Bromion versus Cynosurion* and *Violion*. No development in community LDMC and community SLA was recorded in

Bromion, whereas community SLA sharply increased in *Cynosurion* and *Violion* and community LDMC decreased in *Violion*. Community values of SLA were at the end of June, in the case of the long-time grazed pastures of *Cynosurion* and *Violion*, higher than in the case of the abandoned *Bromion*. Community LDMC showed this pattern in reverse. These results are consistent with those of other studies (Louault et al. 2005; McIntyre 2008) where SLA was found to positively correlate with intensity of disturbance whereas LDMC was found to negatively correlate with intensity of disturbance.

Moreover, *Bromion* showed a significant decrease in the community forage value whereas this value remained stable or even increased in *Cynosurion* and *Violion*, respectively. This was because of changes in plant species composition – spring dominants *Poa pratensis* and *Dactylis glomerata*, with high forage indicator values, significantly decreased while *Brachypodium pinnatum*, with little forage indicator value, substantially increased in *Bromion*. In contrast, *Knautia kitaibelii* with little forage indicator value significantly decreased and *Agrostis capillaris* and *Heracleum sphondylium* with intermediate forage indicator values and *Trifolium repens* (the latter two almost significantly), with high forage indicator value, increased in *Cynosurion*. Similarly, in *Violion*, spring dominants *Bromus erectus*, *Nardus stricta*, *Festuca rupicola* and *Brachypodium pinnatum*, with little forage indicator values, were replaced by *Agrostis capillaris* and *Festuca rubra* with intermediate forage indicator values and by *Trifolium pratense* and *T. repens* with high forage indicator values. Contrasting values of CSDI indicated different seasonal developments of plant species compositions in the investigated sites: *Violion* had substantially higher community seasonal development than *Bromion*.

We assume that the high degree of community seasonal development, which was indicated by high CSDI in *Violion* and by significant development in community SLA in both *Cynosurion* and *Violion*, together with high forage indicator values of later-developing species were accountable for the remarkable stabilization of forage quality later in the vegetation season in these sites. According to the authors' knowledge, this is the first time that seasonal decline in forage quality deferred by seasonal development of plant species composition within the community has been described. However, it was previously suggested that late-flowering species at an immature stage of reproductive development might moderate the seasonal decline in forage quality (Smith and Jones 1991; Duru 1997). Seasonal development of plant species composition is an intrinsic characteristic of many types of semi-natural grasslands, and the extent of such development is influenced by the diversity of plant functional types that is elevated by intensity of disturbance (Kleyer 1999; Louault et al. 2005). This is clear from the long-time unmanaged *Bromion*, where the early spring dominant *Brachypodium pinnatum* quickly closed the canopy and even increased its dominance later in the season. Thus, forage quality decline at this site may be further explained by the plant ageing process that is connected to an increase in less digestible structural tissues (stems) on the account of metabolic tissues (leaves). Contrary to this, *Cynosurion* and *Violion* sites possess communities that have become established under long-lasting grazing pressures starting each season in May or even earlier. Each year, species with early spring growth are suppressed by defoliation and trampling, therefore species such as *Agrostis capillaris* and *Trifolium repens* requiring higher thermic sum (Bovolenta et al. 2008) are able to coexist and

their young tissues become a significant part of the community biomass later in the season. In *Violion*, the increase in legumes (*Trifolium pratense*, *T. repens*) was particularly responsible for stabilization of forage quality, partly due to their high nitrogen concentrations (Ďurková and Jančovič 2003), partly due to positive effect of high nitrogen concentration tissues on digestion process (Niderkorn and Baumont 2009) and partly due to increasing growth and foliar nitrogen concentrations in neighbouring plants (Spehn et al. 2002).

Conclusions

The remarkably stable forage quality obtained by a late harvest of species-rich grasslands should be added to the list of important functional characteristics of semi-natural grasslands, which were lately synthesized by Sanderson et al. (2004). Future agri-environmental measures should take into consideration that a late harvest without decreased forage quality could be expected in grasslands with high seasonal development of species proportions, at least in the first year after the shift from early spring to summer defoliation management.

As the design of this study did not enable the separation of effects of community type from grazing history, future research based on replicated experimental plots with different management types within each respective community is needed. Also, the use of CSDI as a tool for the prediction of seasonal development of forage quality should be further tested and confronted with seasonal developments of crude fibre and OMD of biomass samples.

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