



Original research

Leaf functional traits of abundant species predict productivity in three temperate herbaceous communities along an environmental gradient



Larissa A. Ivanova^{a,b,*}, Natalia V. Zolotareva^c, Dina A. Ronzhina^{a,b}, Elena N. Podgaevskaya^c, Svetlana V. Migalina^{a,b}, Leonid A. Ivanov^{a,b}

^a Plant Ecophysiology Group, Institute Botanic Garden, Ural Branch, Russian Academy of Sciences (IBG UB RAS), 620144 Ekaterinburg, Russia

^b Tyumen State University, 625003 Tyumen, Russia

^c Vegetation and Mycobiota Biodiversity Laboratory, Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (IPAE UB RAS), 620144 Ekaterinburg, Russia

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ABSTRACT

We tested how functional traits of abundant species affect such community properties as photosynthetic capacity and above-ground biomass as well as to what extent do both intra-specific variation and species replacement influence community productivity. We studied above-ground biomass (AGB), functional structure and leaf traits in three herbaceous communities which were located along a local environmental gradient in the south taiga zone: steppe – meadow – forest understory. Leaf thickness (LT) and leaf mass per area (LMA), rates of photosynthesis (A_{\max}) and transpiration (E), chlorophyll content (C_{ab}/A), mesophyll surface area per leaf area (A_{mes}/A) and rate of CO_2 -transfer through the mesophyll surface (TR_{CO_2}) were all measured at the level of species and communities. AGB decreased along the light and water gradient from steppe community to forest understory. The change in AGB depended neither on species richness nor on total coverage, but was positively associated with both species-averaged and community-weighted means of A_{\max} and A_{mes}/A . Intra-specific variability affected community properties to a lesser extent than replacement of abundant species which belonged to different plant functional types (PFTs): mesophytic or xerophytic grasses, herbs or small shrubs with different mesophyll anatomy. Xerophytic herbs possessed larger values of A_{\max} and A_{mes}/A in comparison to mesophytic ones; herbs with dorsiventral leaves had higher A_{\max} than shrubs; and shrubs differed from herbs and grasses by low values of TR_{CO_2} . Increase in the relative abundance of xerophytic herbs and grasses in the steppe resulted in the enhancement of photosynthetic capacity at the community level, in comparison with meadow and forest understory. We suppose predictive significance of A_{\max} and A_{mes}/A of abundant species for AGB of herbaceous community and suggest an approach based on a ratio of PFTs identified by growth form, ecotype and leaf mesophyll anatomy that will help to predict the photosynthetic capacity and productivity of herbaceous communities.

1. Introduction

Over the last few decades, ecologists and botanists have explicitly shown that global change factors influence biodiversity (Chapin et al., 2000; Parmesan, 2006). Biodiversity alteration, in turn, is thought to influence ecosystem functioning (Grime, 2001; Hooper et al., 2005, 2012; Naeem et al., 2009). However, it is quite difficult to describe the relationship between biodiversity and functional processes in plant communities. Biodiversity may be simply and objectively assessed by the taxonomic identity of species, while the quantitative estimation of ecosystem functional properties is rather complicated and demands not

only species recognition but also the analysis of their features of functional significance (Garnier et al., 2004; Diaz et al., 2004; Hooper et al., 2005; Cadotte et al., 2011). In the field of plant ecology such features are called plant functional traits. They are morphological, physiological or phenological parameters of plants, determined at different levels, ranging from individual cell to whole plant (Violle et al., 2007). Plant functional traits are quite easily measurable, closely related to the main functions of plants, but at the same time they should notably vary with environmental conditions (Cornelissen et al., 2003; Garnier and Navas, 2012). Simultaneous consideration of vegetation change and plant functional traits along environmental gradients provides excellent

* Corresponding author at: Plant Ecophysiology Group, Institute Botanic Garden, Ural Branch, Russian Academy of Sciences (IBG UB RAS), 620144 Ekaterinburg, Russia.

E-mail addresses: Larissa.Ivanova@botgard.uran.ru (L.A. Ivanova), nvp@ipae.uran.ru (N.V. Zolotareva), Dina.Ronzhina@botgard.uran.ru (D.A. Ronzhina), enp@ipae.uran.ru (E.N. Podgaevskaya), fterry@mail.ru (S.V. Migalina), Leonid.Ivanov@botgard.uran.ru (L.A. Ivanov).

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opportunities to gain insight into the relation between species composition and functional properties of communities.

The range of functional traits is virtually infinite therefore we need to know which functional traits can be used to assess and monitor vegetation change in response to environmental change (Gillison, 2013). Instead of laborious measure of a majority of plant traits, it is possible to choose only several substantial parameters, since multiple functional traits co-vary in pairs or even in groups (Chapin, 1993; Grime, 1997; Reich et al., 2003; Wright et al., 2004; Reich, 2014). Leaf traits are often considered as parameters of prime importance for task to specify response of plant species to their environment (Loranger and Shipley, 2010; Garnier and Navas, 2012; Gillison, 2013). Leaf mass per area (LMA) and leaf thickness (LT) may be controlled by many environmental variables such as radiation, water supply, temperature, salinity, nutrients and others (Fonseca et al., 2000; Reich et al., 2003; Poorter et al., 2009). These parameters are linked at the same time with the main physiological characteristics. For instance, LMA was found to correlate with photosynthesis, relative growth rate, leaf life-span and others (Wright et al., 2004; Poorter et al., 2009; Hassiotou et al., 2010; Grady et al., 2013). Three leaf traits – leaf life-span, nitrogen per leaf mass and photosynthetic capacity – were recognized as significant predictors of biomass response to climate warming (Gornish and Prather, 2014). Combination of some leaf traits such as mass based photosynthetic rate, specific leaf area and chlorophyll concentration with changes in soil moisture content was used to predict the relative growth rate for six dominant tree species of a dry tropical forest (Chaturvedi et al., 2011). Thus the functional significance of leaf traits within the context of the entire plant is highlighted during the study of plant responses to environment and their links with plant functional properties as growth and productivity (Gillison, 2013).

Productivity change is the principal marker of dynamics in ecosystems. For herbaceous vegetation, the above-ground biomass (AGB) may be used to estimate net primary productivity, as it has been shown in the temperate grassland steppes (Scurlock et al., 2002). AGB accumulation in grasslands can be linked to the photosynthetic activity of plant leaves since it sets the rate of carbon input in the plants. However, given a large amount of external factors affecting leaf photosynthesis, the relationship between the photosynthesis rate of plant species and productivity of plant community remains unclear. A functional approach to biodiversity analysis can help to reveal the determinants of vegetation productivity. Determining the functional traits of species while accounting for the abundance of the species allows proceeding from the species to the ecosystem level (Garnier et al., 2004) as the effect of species on ecosystem properties will depend on their proportional abundance in a community (Grime, 1998). Ecosystem properties can be predicted from the community weighted means of traits (CWM) (Lavorel et al., 2008; Garnier and Navas, 2012; Gillison, 2013). In the case of herbaceous communities, the above-ground biomass has been shown to correlate with CWM of such leaf traits as leaf area, specific leaf area, leaf dry matter content and leaf nitrogen content (Garnier et al., 2004; Pontes et al., 2007).

The functional approach allows to answer another important question: to what extent do both intra-specific variation and species replacement with environmental changes influence community properties (Ackerly, 2003; Garnier et al., 2004; Cornwell and Ackerly, 2009). Both processes can lead to shifts in CWM of a community, and moreover the replacement of abundant species can cause changes in the functional diversity of a community. Functional diversity as the component of functional structure of a community could be assessed by the ratio of plant functional types (PFTs) (Cadotte et al., 2011; Garnier and Navas, 2012; Gillison, 2013). PFTs are groups of species with similar functional traits and similar responses to environment (Box, 1996; Diaz et al., 2004; Cadotte et al., 2011). Separating plant species to PFTs allows us to proceed from operating of the plenty of species to several groups of species.

The use of CWM in studying community properties has been

successfully applied in species-rich grasslands (Garnier and Navas, 2012). In south taiga, we have studied three herbaceous communities, compactly arranged on the steppe relict slope across a gradient of light and water availability. We have determined the main components of the community functional structure: CWMs of the leaf traits and functional diversity. We have chosen the leaf functional traits thought to be tightly linked with photosynthetic capacity of species – LMA, leaf thickness, A_{mes}/A , chlorophyll content, photosynthesis and transpiration rates. These traits concern different aspects of primary productivity and are known to vary along global and local environmental gradients (Cunningham et al., 1999; Fonseca et al., 2000; Voronin et al., 2003; Wright et al., 2004). The leaf trait A_{mes}/A – the ratio of mesophyll surface area to leaf area – requires a special attention. On the one hand, it is an anatomical feature, but on the other hand A_{mes}/A is informative of the surface area for gas exchange at the pathway of CO_2 from substomatal cavities to the sites of carboxylation (Evans et al., 2009; Terashima et al., 2011). This parameter is useful for estimating the mesophyll conductance (Laik et al., 1970; Terashima et al., 2011), studying the adaptation mechanisms of plants to stress (Nobel and Walker, 1985; Mokronosov, 1981; Pyankov et al., 1999), establishing the ecological properties of plant species (Ivanova, 2014) and examining the response of different PFTs to their environment (Ivanova et al., 2016). The link between structural and functional organization of the leaf is most clearly manifested on the level of A_{mes}/A (Mokronosov, 1981). Thus we hypothesized in our work that: 1) leaf functional traits of abundant species can predict productivity of herbaceous communities along a local environmental gradient, 2) the trends of changes of functional traits may be different on the level of species and communities but intra-specific variation to the lower extent than replacement of species influences the community's properties, 3) grouping of the abundant species into PFTs on the basis of leaf functional traits can be used to predict community productivity.

2. Methods

2.1. Study sites and sample collection

The study was conducted on the steppe relict slope of the bank of the Iset' River near Ekaterinburg (56° 35' N, 61° 03' E) in the Urals region of Russia. The study site is located in taiga, namely, in the subzone of pine-birch forests of forest steppe, where isolated isles of relict steppe of small area situated on the southern slopes are surrounded by forest vegetation (Zolotareva and Podgaevskaya, 2012). The topographic profile crosses the slope top in the south-west direction. It is comprised of three herbaceous communities: steppe, meadow and forest understory vegetation (Table 1, Fig. A.1 in Supplementary material). The upper and middle part of the slope is occupied by "steppe community", namely, petrophytic steppe with dominance of *Stipa pennata* L. and *Echinops ritro* L. s.l. The "meadow community", consisting of a steppe meadow, dominated by *Brachypodium pinnatum* (L.) P. Beauv. and *Trifolium montanum* L., was located downslope and was formed on the place of the former forest. The north-eastern part of the slope was covered by pine forest, where the overstory vegetation was dominated by *Pinus sylvestris* L. We however focused only on the ground layer vegetation, which consisted mainly of herbaceous species, dominated by *B. pinnatum*. Site selection of co-located study areas was purposively undertaken in order to minimize the influence of the different macroclimatic and anthropogenic factors that would afford the opportunity to reveal the trends in community properties mostly due to changes in species composition. The examined communities were arranged along environmental gradients of light and water supply, consistent with their positions on the slope. Light intensity, air and soil temperature decreased but soil moisture increased going from steppe to meadow to forest understory (Table 1). Measurements of environmental variables were performed daily at 12:00 and 18:00 h during all periods of the study in the last half of June 2011. Data on the photosynthetic photon

Table 1

Vegetation analysis and environmental variables in studied communities. Above-ground biomass is presented as a mean and standard error for 10 plots per community. Measurements of environmental variables were performed daily during period of study and are presented as a mean.

Parameter	Community		
	Steppe	Meadow	Forest understory
<i>Vegetation characteristics</i>			
Community type	petrophytic steppe	post-forest meadow	steppe pine forest
Species number	36	63	55
Total coverage of community (%)	102	112	96
Crown density (%)	0	0	50
Above-ground biomass ($\text{g} \cdot \text{m}^{-2}$)	166 ± 16	74 ± 5	43 ± 4
<i>Environmental variables</i>			
Photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			
at 12:00	1995	323	228
at 18:00	2050	1560	234
Relative air humidity (%)			
at 12:00	60	60	57
at 18:00	39	50	43
Air temperature ($^{\circ}\text{C}$)			
at 12:00	24.5	24.0	23.3
at 18:00	34	33	32
Soil moisture (%)	10	30	25
Soil temperature ($^{\circ}\text{C}$)	18.6	15.7	12.6

flux density were obtained by LI-COR quantum sensors (LI-COR, USA). The measurements were taken above the leaf canopy in the steppe and meadow communities and above the understory canopy in the forest (about 20–30 cm from soil surface). The “meadow community” was exposed to higher values of photosynthetic photon flux density in the afternoon because of south-west exposure of the slope (Table 1). Air temperature and relative humidity were measured using thermohygrometer Testo 625 (Testo AG, Germany) at 10 cm height above soil surface. Soil temperature was measured at a depth of 10 cm daily at 12:00 h using thermometer Checktemp 1 (HANNA Instruments, Germany). Soil moisture was measured gravimetrically three times during the period of study (every 3–4 days) and presented as average (Table 1). Samples of 50–100 g of soil were taken from 10 to 15 cm depth, weighed, dried at 105 $^{\circ}\text{C}$ for 24 h and reweighed. There was no meaningful precipitation during this period.

Research was conducted starting in the steppe and moving to the forest end of the profile. Leaf functional traits of abundant species, floristic composition and above-ground biomass (AGB) of communities were measured at the peak of the growing season in the period of mass flowering of dominant and abundant species. Fifteen test plots 50 cm \times 50 cm per community were established at random to analyze their floristic composition and coverage of species. Floristic similarity of communities was assessed using the Sørensen index (Sørensen, 1948) which ranges from 0 to 1. The maximal value of 1 confirms a complete similarity of communities. Maximal species richness was calculated for the meadow. This fact can be attributed to the post-forest origin, ecotone character and the intermediate rate of limiting ecological factors – water and light deficiency. The species composition in the steppe appeared to be most specific; that is related to specific ecological conditions – high water deficiency and high insolation rate, which causes the presence of endemic and relict species pertaining to steppe floristic complex. Ten of these test plots (50 cm \times 50 cm) were used for biomass harvests. AGB from each plot was sorted to species. All samples were dried at 70 $^{\circ}\text{C}$ and weighed. Measurements of leaf functional traits were conducted on plants in the vicinity of the test plots for biomass. Dominant and abundant species, which comprise together at least $\approx 70\%$ of cumulative relative abundance of a community, were

Table 2

A list of parameters and abbreviations used in the text.

Abbreviation	Meaning	Units
	<i>Functional traits</i>	
LMA	Leaf mass per area	g m^{-2}
LT	Leaf thickness	μm
A_{max}	Maximal rate of photosynthesis per leaf area	$\mu\text{mol m}^{-2} \text{s}^{-1}$
E	Transpiration rate per leaf area	$\text{mmol m}^{-2} \text{s}^{-1}$
A_{mes}/A	Ratio of total mesophyll surface area to leaf projection area	–
TR_{CO_2}	The rate of CO_2 -transfer through mesophyll surface area unit	$\mu\text{mol m}^{-2} \text{s}^{-1}$
C_{ab}/A	Chlorophyll content $a + b$ per leaf area	mg dm^{-2}
	<i>Community properties</i>	
AGB	Above-ground biomass	g m^{-2}
abund	Mean value of functional trait for abundant species in the community (subscript av, Trait _{av})	–
dom	Mean value of functional trait for dominant species in the communities (subscript dom, Trait _{dom})	–
CWM	Community-weighted mean of functional trait (subscript CWM, Trait _{CWM})	–
obs	Observed value of functional trait for the whole community using relative contribution of abundant species to above-ground biomass (subscript obs, Trait _{obs})	–
pred	Predicted value of trait for the whole community using ratio of PFTs in coverage of community (subscript pred, Trait _{pred})	–

sampled so that 10–11 plant species were analyzed in each community. Leaves for anatomical and biochemical analysis were harvested from the middle leaf tier of 20 fully developed individuals of each species and stored in a refrigerator for two to three hours.

2.2. Leaf traits measurements

We measured traits from different levels of leaf organization: structural traits of the whole leaf, physiological and biochemical parameters per leaf area unit and mesophyll and cell parameters (Table 2). To measure leaf mass per area (LMA) leaf discs of known diameter (or whole leaf blades in the case of small or narrow leaves) were taken from 10 to 20 individuals of each species, photographed, dried at 75 $^{\circ}\text{C}$, and weighed. Leaf thickness (μm) was measured using a PK-1012E digital micrometer (Mitutoyo, Japan) for ten leaves per species. Content of chlorophyll $a + b$ per leaf area (C_{ab}/A) was measured for 10 individuals of each species from leaves frozen in liquid nitrogen. Pigments were extracted with 80% acetone and measured using the Odyssey DR/2500 portable spectrophotometer (Hach, USA). C_{ab}/A was calculated according to Lichtenthaler and Wellburn (1983). Maximum photosynthetic rate (A_{max}) and transpiration rate (E) were measured in the first half of the day – from 10:00 till 14:00–on intact mature leaves of three individuals in each species per each community using infra-red gas analyzer Li-6400xt (LI-COR, USA) under ambient CO_2 concentration, relative humidity of the air 35%, controlled leaf temperature 22 $^{\circ}\text{C}$ and photosynthetic photon flux density (PPFD) 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Data on A_{mes}/A were obtained according to the method of leaf mesostructure (Ivanova, 2014) using leaf tissue maceration. Between ten and twenty leaf discs or whole small leaves from 10 to 20 plants (1 leaf per plant), which were fixed previously in a 3.5% solution of glutaraldehyde, were taken for analysis. The number of cells per leaf area unit was determined in 20 replicates in cell suspension using a hemocytometer “Goryaev chamber” (Minimed, Bryansk, Russia) after maceration of leaf pieces of known area (ca. 1 cm^2) in 2–3 ml of 20% KOH under heating up to 90 $^{\circ}\text{C}$ (described in detail by Ivanova and Pyankov, 2002). Cell sizes were determined in 30 replicates in cell suspension after maceration of leaf pieces in 1 N HCl under heating up to 40–50 $^{\circ}\text{C}$. The mesophyll cell volume and cell

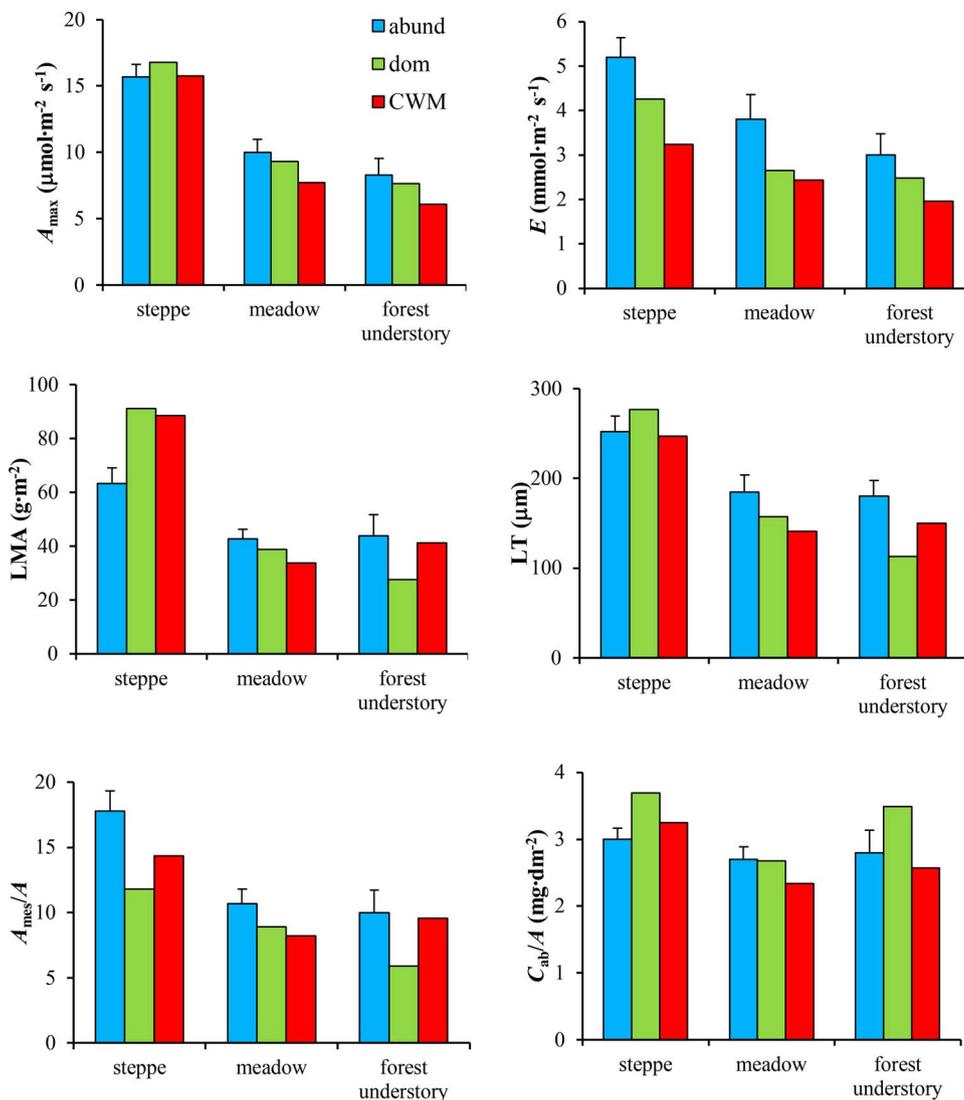


Fig. 1. Leaf functional traits at the level of species and communities: abund – species-averaged value for abundant species, dom – species-averaged value for dominant species, CWM – community-weighted mean. X-axis presents studied communities: steppe, meadow, forest understory.

surface area separately for each type of photosynthetic tissue (palisade and spongy, segmented mesophyll cells of grasses) were calculated according to the projection method (Ivanova and Pyankov, 2002). The total cell surface area per leaf area unit (A_{mes}/A) was determined by multiplying the average cell surface area by the cell number per leaf area. The rate of CO_2 -transfer through mesophyll surface area (TR_{CO_2}) was calculated by dividing the maximum rate of CO_2 -uptake per leaf area (A_{max}) by A_{mes}/A .

2.3. Plant functional types (PFT)

Grouping into PFTs was based on traditional qualitative characteristics of species: growth form (shrub, perennial herb, annual herb), systematic (monocot, dicot), leaf habit (deciduous, evergreen) (Reich et al., 2003; Gillison, 2013). Given the known effect of leaf anatomy on functional properties of species (Gillison, 2013) we used general mesophyll anatomy for grouping PFTs. While doing it, we primarily relied on the location and structure of mesophyll inside the leaf – homogenous, dorsiventral, isopalisade, graminoid. Dorsiventral (DV) and isopalisade (IP) types are usual for dicots. In the first type, the upper layer of mesophyll takes form of palisade cylindrical cells while the lower layers consist of spongy round or irregular cells. IP type has palisade tissue, both on upper and lower layers. Graminoid type was determined for grasses (Poaceae) and sedges (Cyperaceae). It was characterized by a large volume of vascular tissue and a limited location of

mesophyll between vascular bundles. Graminoid mesophyll consisted of segmented or irregular cells. In addition, taken into account the significant functional differences between ecotypes of herbaceous plants (Ivanova 2014), we set specific kinds of functional groups – “xerophytic” and “mesophytic”. Ecological characteristics and phytocenotypes for plant species are presented according to Kulikov (2005).

2.4. Data analysis

Using functional traits of species and their contribution to community we calculated several parameters for functional trait at the community level (Table 2). First, average values of traits were calculated as the mean for both dominant and abundant species in each community. Second, community-weighted mean of functional trait ($\text{Trait}_{\text{CWM}}$) was calculated as described in Garnier et al. (2004):

$$\text{Trait}_{\text{CWM}} = \sum_{i=1}^n m_i \times \text{trait}_i$$

where m_i is the proportion of species i in above-ground biomass of community, n is the number of abundant species in community, trait_i is the trait value of species i in the current community. Third, we determined the observed and predicted values of functional traits for the whole community. Observed value of community trait was calculated as: $\text{Trait}_{\text{observed}} = \frac{\text{Trait}_{\text{CWM}}}{m_{\text{abund}}}$, where m_{abund} is the cumulative proportion of abundant species to above-ground biomass of community. Predicted value of functional traits for the whole community was determined using the ratio of PFTs in the total coverage of

community, i.e. as: $Trait_{predicted} = \sum_{j=1}^k p_j \times trait_j$, where p_j is the proportion of PFT j in the total coverage of community, k is number of PFTs in community, $trait_j$ is the mean value of a trait for species of this PFT.

To test for differences between trait mean values of PFTs, after using one-way ANOVA we relied on the Tukey-test. To compare the species-specific and environmental effects, we used two-way ANOVA. Differences were considered to be significant at $p \leq 0.05$. In figures and tables, means \pm SE are presented. Different letters on figures and tables indicate significant differences.

3. Results

Studied communities markedly differed in AGB values, which were maximal in the steppe, two-fold lower in the meadow and four-fold lower in the forest understory (Table 1). AGB were not linked with total coverage or species richness in the community. Steppe community had maximal AGB even though species richness was minimal in this community. Total coverage for all of the communities was similar. In contrast, means of leaf traits changed along the gradient both at the level of species and of communities (Fig. 1). Photosynthetic capacity declined from the steppe to meadow to forest understory and had the same values for respective community regardless of the calculation method. Transpiration rate decreased in the same direction, but to a lesser degree than A_{max} . Values of LMA as well as LT were higher in the steppe than in the other communities. Foliage of the steppe community was characterized by a two-fold larger ratio of mesophyll surface area to leaf projection area (A_{mes}/A) in comparison with that of meadow and forest understory. Chlorophyll $a + b$ content per leaf area did not show significant differences between communities.

Taxonomic composition of communities significantly differed despite their proximity on the same slope. Meadow and forest understory were the closest – Sørensen index for this pair was 0.59. These have the same dominant species – *Brachypodium pinnatum*. For the steppe–meadow pair, the Sørensen index reached 0.44. The steppe and forest understory had the largest differences in their taxonomic composition (Sørensen index 0.37). The replacement of abundant species could be clearly traced (Table 3). Only two species had essential abundance in all three communities, *Echinops ritro* and *Chamaecytisus*

ruthenicus. We have analyzed the intra-specific changes of leaf functional traits for these two and seven more species, which were studied in any two communities (Fig. 2). There was a small effect of intra-specific changes on leaf traits variation along the environmental gradient. LMA and LT depended by 80–90% on plant species and only by 10% on species variability. A_{mes}/A did not significantly depend on growing conditions and was mainly species-specific. Variation of A_{max} along environmental gradient inside species was about 20% from the total variation but the direction of A_{max} changes differed, depending on the species. In some species, A_{max} was maximal in steppe or in meadow, whereas other species had stable rate of A_{max} , regardless of the growing conditions (Fig. 2).

Functional diversity of community changed along the gradient of water and light availability. From 15 PFTs, which overall could be distinguished among the studied communities, only 6 PFTs had significant contribution to the total coverage of communities (Fig. 3). The functional structure was similar in the meadow and forest understory – in both communities mesophytic grasses and mesophytic herbs with dorsiventral leaf anatomy dominated. In the steppe, the proportion of xerophytic grasses and herbs with isopalisade leaves increased by several times. Main differences between PFTs existed in traits related to photosynthetic capacity (Table 4). Xerophytic herbs were characterized by higher values of A_{mes}/A and A_{max} than mesophytic ones. Dicotyledonous herbs with isopalisade leaves had larger values of these traits than plants with dorsiventral anatomy. Shrubs differed from herbs and grasses by low values of TR_{CO_2} while herbs and grasses did not significantly differ in this parameter. The lowest TR_{CO_2} was found in the evergreen leaves of dwarf shrub *Vaccinium vitis-idaea*.

Finally, AGB was related to leaf functional traits of abundant species of communities. In the steppe community, which is characterized by the highest value of AGB, plant species with high photosynthetic capacity predominated. This fact remained at any manner of calculations, both as arithmetic average for abundant species, as well as weighted means (Figs. 1 and 4). The mean value of A_{mes}/A for abundant species was also positively related to the AGB of a community. Conversely, the community of forest understory had the lowest AGB and included mainly plant species with low values of A_{max} and A_{mes}/A . Predicted values of A_{max} per unit of community area, obtained using the ratio of PFTs in total coverage, showed a good convergence with the observed values of

Table 3

A list of studied plant species, their characteristics and relative abundance in community (coverage, %): 1–steppe, 2–meadow, 3–forest understory. GF – growth form, HP – herb perennial, S – shrub, DSeg – dwarf shrub evergreen; ct – coenotype, f – forest, fe – forest edge, fs – forest-steppe, fm – forest-meadow, ms – meadow-steppe, ps – petrophytic-steppe, s – steppe; TM – type of mesophyll, DV – dorsiventral, GR – graminoid, IP – isopalisade; eg – ecological group, m – mesophyte, mx – mesoxerophyte, xm – xeromesophyte, x – xerophyte. *this species was not studied in this community.

Plant species	Family	GF, ct	TM, eg	Community		
				1	2	3
<i>Astragalus danicus</i> Retz.	Fabaceae	HP, fs	DV, xm		5	0.5*
<i>Brachypodium pinnatum</i> (L.) P. Beauv.	Poaceae	HP, fm	GR, m		40	35
<i>Calamagrostis arundinacea</i> (L.) Roth	Poaceae	HP, fm	GR, xm		2	5
<i>Calamagrostis epigejos</i> (L.) Roth	Poaceae	HP, fs	GR, mx	1	9	
<i>Carex caryophyllea</i> Latourr.	Cyperaceae	HP, fs	GR, xm	3	1*	0.01*
<i>Carex montana</i> L.	Cyperaceae	HP, f	GR, xm		3	0.5*
<i>Chamaecytisus ruthenicus</i> (Fischer ex Woloszczak) Klásk.	Fabaceae	S, fe	DV, xm	3	2	6.5
<i>Echinops ritro</i> L. s.l.	Asteraceae	HP, ps	DV, x	27.5	3	8
<i>Onosma simplicissima</i> L.	Boraginaceae	HP, ps	DV, mx	0.5		
<i>Phleum phleoides</i> (L.) H.Karst.	Poaceae	HP, ms	GR, xm	0.5	1	
<i>Potentilla humifusa</i> Willd. ex Schldtl.	Rosaceae	HP, s	DV, mx	3	0.01	0.01
<i>Psephellus sibiricus</i> (L.) Wagenitz	Asteraceae	HP, ps	IP, xm	6.5	0.5*	0.5
<i>Rubus saxatilis</i> L.	Rosaceae	HP, f	DV, m		0.5	4
<i>Seseli krylovii</i> (V.N. Tikhom.) Pimenov and Sdobnina	Apiaceae	HP, fe	DV, xm		1*	3
<i>Stipa pennata</i> L.	Poaceae	HP, s	GR, mx	30	0.01*	
<i>Trifolium montanum</i> L.	Fabaceae	HP, fs	DV, xm		17.5	2
<i>Vaccinium myrtillus</i> L.	Ericaceae	S, f	DV, m			0.5
<i>Vaccinium vitis-idaea</i> L.	Ericaceae	DSeg, f	DV, m		1*	1
<i>Veronica spicata</i> L.	Scrophulariaceae	HP, ms	IP, xm	1	0.01*	
<i>Vincetoxicum hirundinaria</i> Medik.	Asclepiadaceae	HP, fs	DV, xm	0.5	0.01*	

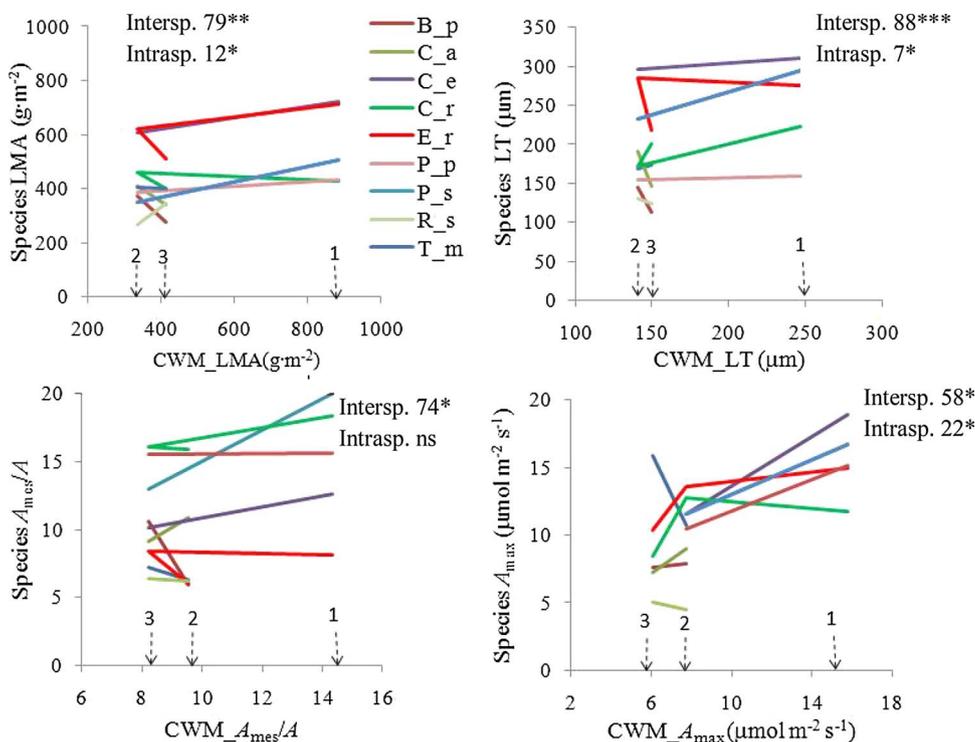


Fig. 2. Intra-specific variation of leaf functional traits among studied communities. X-axis presents values of community-weighted means of functional traits in three studied communities: 1–steppe, 2–meadow, 3–forest understory. Y-axis presents the actual trait values of plant species: B_p – *Brachypodium pinnatum*, C_a – *Calamagrostis arundinacea*, C_e – *Calamagrostis epigeios*, C_r – *Chamaecytisus ruthenicus*, E_r – *Echinops ritro*, P_p – *Phleum phleoides*, P_s – *Psephellus sibiricus*, R_s – *Rubus saxatilis*, T_m – *Trifolium montanum*. The results of two-way (main effects) ANOVA are presented: Intersp. – the contribution of interspecific differences (species-specific effect) in trait variation (%), Intrasp. – the contribution of intra-specific variation (environmental effect) (%).*, $p \leq 0.05$; ***, $p \leq 0.001$; ns – not significant.

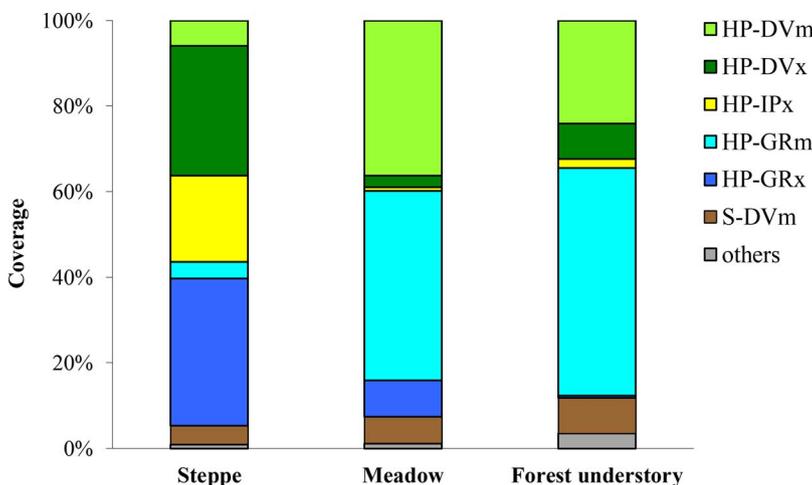


Fig. 3. Ratio of PFTs in the total coverage of studied communities. HP-DVm – mesophytic dicotyledonous herb perennial with dorsiventral mesophyll, HP-DVx – xerophytic dicotyledonous herb perennial with dorsiventral mesophyll, HP-IPx – xerophytic dicotyledonous herb perennial with isopalisade mesophyll, HP-GRm – mesophytic monocotyledonous herb perennial with graminoid type of mesophyll, HP-GRx – xerophytic monocotyledonous herb perennial with graminoid type of mesophyll, S-DVm – mesophytic shrub with dorsiventral mesophyll, others – others plant functional types.

Table 4

Leaf functional traits of different PFTs. GF – growth form, HP – herb perennial, S – shrub, DS – dwarf shrub, eg – evergreen; Syst. – systematic position, Dicot. – dicotyledonous, Mon. – monocotyledonous; TM – type of mesophyll, DV – dorsiventral, GR – graminoid, IP – isopalisade; Ecol. – ecological group, m – mesophytic, x – xerophytic. Results of one-way ANOVA are presented in the bottom of the table and p -level of F -criteria. **, $p \leq 0.01$, ns – not significant.

PFTs traits				PFT	Functional traits		
GF	Syst.	TM	Ecol.		A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	A_{mes}/A	TR_{CO_2} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
Herbaceous	Dicot.	DV	m	HP-DVm	$8.8 \pm 1.4\text{b}$	$7.8 \pm 0.7\text{b}$	$1.20 \pm 0.20\text{a}$
Herbaceous	Dicot.	DV	x	HP-DVx	$14.5 \pm 1.8\text{a}$	$17.1 \pm 1.5\text{a}$	$1.00 \pm 0.11\text{a}$
Herbaceous	Dicot.	IP	x	HP-IPx	$16.1 \pm 2.5\text{a}$	$18.8 \pm 3.6\text{a}$	$0.89 \pm 0.13\text{a}$
Herbaceous	Mon.	GR	m	HP-GRm	$9.2 \pm 1.1\text{b}$	$11.1 \pm 1.7\text{ab}$	$0.86 \pm 0.08\text{a}$
Herbaceous	Mon.	GR	x	HP-GRx	$15.8 \pm 2.4\text{a}$	$14.1 \pm 0.8\text{a}$	$1.20 \pm 0.20\text{a}$
Woody	Dicot.	DV	m	S-DVm	$11.0 \pm 1.3\text{ab}$	$16.8 \pm 0.8\text{a}$	$0.65 \pm 0.08\text{b}$
Woody	Dicot.	DV	m	DSeg-DVm	$3.8 \pm 0.4\text{c}$	$22.1 \pm 2.4\text{a}$	$0.19 \pm 0.02\text{c}$
F- criteria					4.3**	4.4**	ns

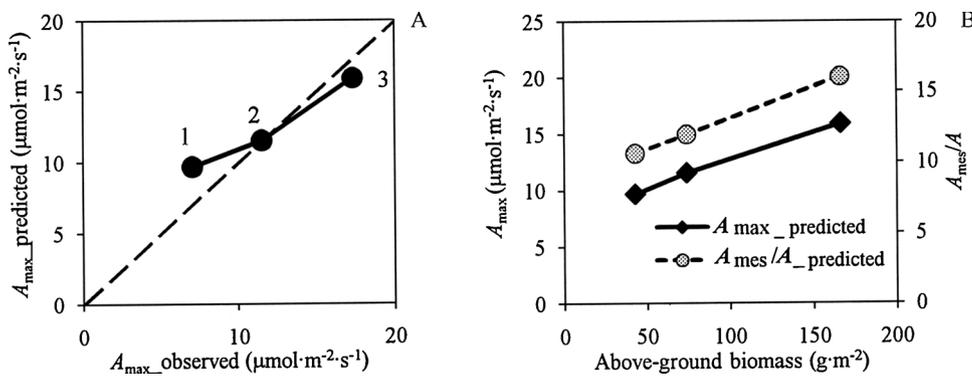


Fig. 4. The link between observed and predicted (using PFTs ratio in community) values of photosynthetic capacity of community (dashed line presents ratio of values 1:1) (A) and a link of above-ground biomass with predicted values of communities functional properties (B).

this parameter, which were obtained using CWM of A_{\max} and summarized contribution of abundant species to community's AGB (Fig. 4A). We have also found a positive relationship between the AGB and the predicted values of A_{mes}/A and A_{\max} for community canopy (Fig. 4B).

4. Discussion

4.1. Association of leaf mesophyll traits of abundant species with communities' productivity

Community's productivity is determined by both photosynthetic activity and projective coverage of the leaf canopy. Our findings indicate that while the studied communities differed in AGB in the magnitude of 2–4 times, they had a similar coverage (Table 1). These results demonstrate that productivity of herbaceous vegetation along the studied gradient did not depend on canopy size. AGB was neither influenced by species richness. These observations are consistent with the idea that community properties are determined mainly by species traits rather than by species' number (Chapin, 1993; Grime, 1997, 2001; Diaz et al., 2004; Garnier et al., 2004; Cadotte et al., 2011). For example, there were positive relationships of the ecosystem above-ground net primary productivity with the species-averaged relative growth rate (RGR) (Chapin, 1993) and the community-weighted LMA (Garnier et al., 2004). Both parameters – RGR and LMA – are, in turn, often associated with the net photosynthesis rate (Evans, 1972; Lambers and Poorter, 1992). In other cases, the biomass of plant species did not associate with their LMA but at the larger extent was related either to the foliar nitrogen content (Pontes et al., 2007) or directly to A_{\max} (Gornish and Prather, 2014). Our results showed an association between the AGB of communities and both species-averaged and community-weighted means of A_{\max} . Searching other leaf functional traits linked to plant adaptation ability and biomass response may improve the predictive significance of A_{\max} (Gornish and Prather, 2014). Since A_{mes}/A is also related to AGB (Fig. 4) this parameter may elevate the effectiveness of predicting the community's productivity, especially as long as values of A_{mes}/A are more stable within species under different growth conditions, compared to A_{\max} (Fig. 2). The analysis among all plant species showed a general positive relationship between A_{\max} and A_{mes}/A ($r = 0.37$, $p < 0.05$). A similar positive association between the mesophyll surface area and the assimilation rate or mesophyll conductance was found by other authors (Nobel and Walker, 1985; Terashima et al., 2011). This confirms an important role of A_{mes}/A as a structural constraint of the net photosynthesis rate. High values of E in the steppe plants also indicate that not stomatal, but mesophyll diffusion resistance makes substantial contribution to the maintenance of A_{\max} . However stomatally controlled mechanisms can also play a substantial role in CO_2 diffusion inside the leaf canopy in natural ecosystems (Evans et al., 2009; Loranger and Shipley, 2010; Launiainen et al., 2016). Future studies might profitably include leaf stomatal traits when leaf traits are used for assessment of AGB changes. In this study, at the

community-level, higher community's A_{\max} in the steppe corresponded to higher community's A_{mes}/A . This demonstrates a direct link between photosynthetic capacity of community and photosynthetic tissue architecture inside leaf canopy. Since chlorophyll content per leaf area did not differ between communities, high level of A_{\max} of steppe species must be caused mainly by the diffusive component of mesophyll conductance. We suppose that the main reason behind high A_{\max} in steppe plants is large A_{mes}/A that facilitates CO_2 diffusion inside leaves. The strong positive correlation between community-level means of A_{\max} and A_{mes}/A with AGB in our study confirms that these leaf traits are good predictors of productivity of herbaceous communities, arranged along contrasting light and water gradient.

4.2. Changes of leaf functional traits along environmental gradient

Our findings suggest significant changes in leaf traits along an environmental gradient both at the species and at the community levels. At the species level, changes in leaf traits with irradiance and water deficit indicate the replacement of species with thin leaves, low values of LMA and A_{mes}/A by species with thicker leaves, high LMA and A_{mes}/A . The same trends in changes of these parameters along the gradient remained at the community level. The coincidence of trends in the species-averaged and weighted means along the gradient confirms that traits of most abundant species could represent functional properties of the entire community (Garnier et al., 2004; Castro-Díez, 2012). Moreover, the most robust differences in leaf traits were found between dominants of communities (Fig. 1).

The increase in CWM values of LMA and LT along gradient is consistent with the common opinion about leaf features of plants from drier and well illuminated sites (Cunningham et al., 1999; Fonseca et al., 2000; Ackerly, 2003; Wright et al., 2004; Ivanova, 2014). At the community level, it is consistent with data about the positive relation of community-level values of LMA with insolation (Ackerly, 2003). The study of 22 herbaceous plant communities along the global aridity gradient also revealed a positive relation of species-averaged means of LMA with annual temperature and a negative relation with rainfall (Ivanov et al., 2009). The increase of LMA along gradient of water deficit is thought to be associated with the enhancement of the proportion of non-photosynthetic tissues in the leaf (Vorontin et al., 2003; Poorter et al., 2009). However, we found a positive correlation between LMA and the sizes of photosynthetic tissue expressed in values of A_{mes}/A among all samples ($r = 0.42$, $p < 0.01$). In this case, the increase of LMA is related not only to the rise in proportion of supporting and protective tissues but also to the increase in the structural complexity of the mesophyll (Ivanova, 2014; Yudina et al., 2017; Peguero-Pina et al., 2017). Denser stacking of the mesophyll cells is reflected in high A_{mes}/A that results in the increase of the surface area for gas diffusion elevating internal conductance for CO_2 . In the forest understory, plant species had low values of A_{mes}/A which corresponds to adaptation of their photosynthesis to shading (Terashima et al., 2011; Ivanova, 2014). The meadow community is characterized by the predominance of species

with intermediate values of A_{mes}/A and photosynthetic capacity. Despite the maximum species richness in the meadow, we have observed the same level of variation in functional traits inside the meadow species. This points to the same extent of natural selection pressure in conditions of the meadow as of the steppe and forest. Steppe plants, characterized by high LMA and A_{mes}/A , reached higher levels of both A_{max} and E . Thus, such xerophytic high-LMA leaves would present higher photosynthetic capacity per leaf area but only in the absence of critical water deficit, i.e. at low stomatal resistance. Studied communities are located in south taiga in temperate climate conditions therefore mesophyll conductance prevailed over stomatal constraints of CO_2 uptake. In other climatic conditions stomatal control may be much more significant (Buckley et al., 1980).

4.3. Intra-specific trait variation or replacement of species with different functional traits

Changes in community-level means may be caused by intra-specific changes of traits, or by the replacement of species with different functional traits, or due to both processes (Garnier et al., 2004). Relationships between both processes of phenotypic plasticity and species replacement are worth considering in the context of climate change (Cornwell and Ackerly, 2009). The first response to climate change is phenotypic plasticity. Secondly, species replacement occurs (Cornwell and Ackerly, 2009). Similarly, in the studies with experimental climate warming, we initially observed changes in leaf functional traits within individual species as soon as during the first growing season (Ivanova et al., 2016). After several years of treatment, shifts in the abundance of species and species replacement appear (Hollister et al., 2015). Phenotypic plasticity can contribute to community properties at local-scale gradients (Cornwell and Ackerly, 2009; Kumordzi et al., 2015), i.e. in close communities. At the same time, the replacement of species with other functional traits is more important under contrasting conditions (Ackerly, 2003; Cornwell and Ackerly, 2009; Garnier and Navas, 2012). In agreement with other findings at community level (Garnier et al., 2004; Pontes et al., 2007) our investigation showed that the intra-specific variation in leaf traits was lower than differences between species. In particular, 70–80% of variation in structural leaf traits could be explained by the species factor (Fig. 2). Thus, intra-specific variation could explain the changes in the meadow–forest understory pair, which are the most similar in species composition due to their common origin and similar ecological conditions. These communities possess the same functional composition: mesophytic herbs with dorsiventral leaf anatomy and mesophytic grasses dominated in both communities. There were only low shifts in CWMs of leaf traits from forest understory to meadow.

The ratio of PFTs essentially changed in the steppe – the proportion of xerophytic grasses and herbs with isopalisade leaves increased by several times. This led to the jump in values of leaf traits in the steppe in comparison with the meadow and forest understory because of significant differences between PFTs in leaf traits especially such as A_{mes}/A and TR_{CO_2} . These mesophyll parameters are effective under scaling from cell-level to the level of community by the analysis of photosynthetic capacity and productivity of community. Both A_{mes}/A and TR_{CO_2} could also be useful in the analysis of communities with higher proportion of woody and evergreen species than in the present study (Table 4) because of clear differences between herbaceous and woody plants in mesophyll structure and functioning (Ivanova, 2012). Thus, the replacement of abundant species belonging to different PFTs appears to be the greatest reason for changes in community's properties.

The use of mesophyll structure as additional parameters for grouping into PFTs allowed us to propose an approach to predicting the productivity of herbaceous community. Predicted values of photosynthetic capacity, which were based on mean mesophyll traits and relative abundance of PFTs, showed a good convergence with the observed data obtained from A_{max} of each species and contribution of

species to the biomass of community (Fig. 4). The analysis of other leaf traits did not detect a clear link of AGB with either biochemical (pigment content) or morphological (LMA, LT) parameters but revealed a relationship between AGB, abundant species-averaged means and CWM of A_{max} and A_{mes}/A (Fig. 4, A.2 in Supplementary material). This circumstance means that leaf traits at the mesophyll-level, taken for abundant species, could predict productivity of a herbaceous community. The application of this approach allows not only to improve predicting the response of plant communities but also to provide the understanding of mechanisms of regulation of the CO_2 gas exchange by the leaf canopy of vegetation in a changing climate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.flora.2017.11.005>.

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