Ecol Res (2014) 29: 607–617
DOI 10.1007/s11284-014-1148-z

ORIGINAL ARTICLE

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Leaf-trait responses to environmental gradients in moorland communities: contribution of intraspecific variation, species replacement and functional group replacement

Received: 13 November 2013 / Accepted: 1 April 2014 / Published online: 1 May 2014

Abstract The values of many important traits of plants in a community change along environmental gradients. Such changes may involve intraspecific variation and replacement by species that have different trait values. We hypothesized that they also involve the variation within and among functional groups (FGs) to the environmental dependence of trait values at the community level. We studied environmental dependence of trait values in 27 moorlands at various scales and analyzed to what extent intraspecific variation, species replacement within FGs and FG replacement contribute to the gradient of community trait values. The community structure in moorlands was influenced mainly by two environmental factors: temperature and water condition. Plants inhabiting sites with low temperature and low-pH generally tended to have lower maximum leaf height, greater leaf mass per area, and smaller leaf size. At the community level, site-mean of maximum leaf height and leaf size generally increased with increasing temperature and water pH. Our analysis demonstrated that the relative contributions of intraspecific variation, species replacement within FGs and FG replacement differed depending on combinations of the traits and environments. The contribution of FG replacement varied considerably among cases (0.6–34.5 %). Species replacement within FGs, which has received little attention in previous studies, was most responsible for the community-level changes (31.6–65.3 %) and intraspecific variation also made a large contribution (22.9–57.9 %). Understanding such various mechanisms involving intraspecific variation and species replacement should help us better predict how the structure and functioning of moorland plant communities will respond to climate change.

Keywords Functional diversity · Functional traits · Growth form · Leaf habit · Altitude · Leaf height · Leaf mass area · Leaf size

Introduction

In plants, the physiological and morphological traits that are related to resource acquisition and use influence their fitness. Many such traits differ quantitatively among species. Previous studies have shown that the values of several important traits of plants in a community change along environmental gradients (e.g., McGill et al. 2006; Westoby and Wright 2006). This evidence implies that only species that have a trait value within a certain range can survive in the environment (environmental filtering) and that the successful range differs depending on environmental factors.

Changes in trait values along an environmental gradient may involve plastic responses to environmental change (intraspecific variation) and replacement by species that have different trait values (interspecific variation), both of which result in changes in the community traits related to ecosystem functions along an environmental gradient (Ackerly 2003; Ackerly and

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Electronic supplementary material The online version of this article (doi:10.1007/s11284-014-1148-z) contains supplementary material, which is available to authorized users.

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Cornwell 2007; Cornwell and Ackerly 2009). Cornwell and Ackerly (2009) analyzed the community assembly across the varied topography of a coastal California landscape consisting of a mosaic of woody vegetation. They found that the community mean of leaf mass per area (LMA) and the community mean of individual leaf area decreased with decreasing soil water content, and intraspecific shifts in functional traits were small relative to the changes that could occur after species replacement. These results imply that higher LMA and smaller leaf area are favoured in dry environments, and species that have greater plasticity in these traits can tolerate a greater range of water availability.

The concept of plant functional group (FG) has been used to classify vegetation or plant species based on plant functions. This classification is useful when the functional difference is discrete: for example, presence of wintering leaves (evergreen or deciduous) and stems (shrubs or herbs), and physiological differences (C3/C4/CAM and nitrogen fixation). Such grouping assumes that plants in the same group have a common response to the environment (Rusch et al. 2003). It has been shown that many functional traits differ considerably among FGs; for example, evergreen species have higher LMA, lower leaf nitrogen concentration and longer leaf life span than deciduous species (Reich et al. 1995; Diaz et al. 2004; Wright et al. 2005). Therefore variation in community traits may be explained mainly by species replacement occurred across FGs. On the other hand, since there often be a large variation in trait values within FG (Wright et al. 2005), variations within species or within FG may also be important for community traits. Here we provide an additional framework for assessing the contributions of FG replacement and within-FG variation, as well as species replacement and intraspecific variation, to community assembly. Clarifying how such different attributes sorted into FGs affect the relationships between environmental factors and community traits would improve our understanding of community assembly processes and lead to better predictions of community responses to environmental factors (Ordoñez et al. 2010). Previous studies showed that FG composition is affected by environmental factors such as altitude, latitude and the length of time favourable for growth (Kikuzawa 1991, 1995; Vazquez and Givnish 1998; Sieben et al. 2010), the light environment (Ackerly et al. 2002), and traits related to topography such as soil nutrient levels and water condition (Eckstein et al. 1999; Spasojevic and Suding 2012). Ackerly et al. (2002) investigated the distribution of chaparral shrub species in relation to the variation in leaf traits along a light gradient. They found that community-level leaf size and SLA declined with increasing insolation because evergreen species with thicker and smaller leaves preferentially occupied more exposed sites, whereas deciduous species preferentially occupied more shaded sites. To date, however, no study has investigated quantitatively how much FG-level replacement and trait variation within-FGs contribute to the change in community-level traits along environmental gradients relative to the contributions of species-level replacement and trait variation.

A moorland is a type of wetland established where acid peat has accumulated for a long period. Moorland vegetation has high species diversity representing various FGs (Mochida et al. 1997; Kamiyama et al. 2010), often with a large number of endemic and threatened species (Wahren et al. 1999; Lienert et al. 2002; Galeuchet et al. 2005; Hájková et al. 2006). Moorlands are generally found in regions with cool climates, because the slower decomposition due to low temperature allows for the accumulation of peat. Therefore, moorlands are considered to be vulnerable to environmental change, especially to global warming (Gorham 1991; Chapin et al. 2000). Moorlands generally possess different vegetation types, bog and fen, even in one site depending on the hydrology, pH and trophic state (Schimper 1903; Wheeler and Proctor 2000).

Many studies have shown that the species composition of moorlands changes along environmental gradients (e.g., Malmer 1986; Økland et al. 2001; Tahvanainen 2004). For example, Hájková et al. (2006) showed that altitude and water mineral richness affected species composition in wetlands in the Bulgarian high mountains. Sieben et al. (2010) studied the distribution of plant FGs across altitudinal and wetness gradients in wetlands of South Africa. They showed that graminoids were more prominent at lower altitudes, whereas forbs and rosette plants, which were largely absent at the lower sites, were more prominent at higher altitudes. However, it remains unclear how such changes in species composition affect trait values at the community level.

The aim of the present study was to elucidate the functional linkage between species composition, FG composition and environmental factors in moorland ecosystems in northern Japan. We investigated the following two questions: (1) how do leaf trait values change along environmental gradients? and (2) to what extent do intraspecific variation, species replacement within FGs and FG replacement contribute to the gradient of community trait values? We studied 27 moorlands across gradients of environmental factors, including altitude, temperature, pH, and electric conductivity, which enabled us to assess the multivariate relationships between species composition and environmental factors. We measured three representative leaf traits, leaf height, LMA and individual leaf size, which are important traits for plant and ecosystem functioning (Westoby 1998; Milla and Reich 2011).

Materials and methods

Study area

The study sites were established in the Hakkoda Mountain range [the highest point is located at 40°39′N, 140°52′E, 1584 m above sea level (a.s.l.)], Aomori Prefecture, northern Honshu, Japan. There are many moorlands in this area, most of which were created in depressions in the layer of volcanic ash that settled on the ground following a huge eruption in the Pleistocene (Muraoka and Takakura 1988; Koike et al. 2005). Pollen analyses suggested that the species composition of the moorlands has been maintained for 1000–2000 years (Yonebayashi 1996, 2001; Yoshida 2006). We investigated 27 moorlands, each of which was isolated from other moorlands by forests or shrubs (Supplementary Table A1). Species composition and diversity were described in our previous studies (Sasaki et al. 2012a, b; 2013).

Vegetation sampling

We established six 20-m transects distributed evenly within each moorland site and laid out five 1 × 1 m quadrats on each transect at intervals of 5 m, for a total of 810 quadrats along 162 transects sampled at 27 sites in the study area. In August and September 2009, the cover of each species in each quadrat was visually estimated by using a modified Daubenmire percent cover scale: 1, <1 %; 2, 2–5 %; 3, 6–25 %; 4, 26–50 %; 5, 51–75 %; 6, 76–95 %; and 7, >95 %. The cover of each species was determined by converting the Daubenmire scale to the midpoint of the cover range. We excluded all mosses and pteridophytes from analyses.

Two of the six line transects at each site were randomly chosen for measurements of plant morphological traits. Maximum leaf height, LMA and leaf size were determined in each species emerging in each transect, except for woody seedlings and species that were too rare to allow for replication, following protocols described by Cornelissen et al. (2003). Maximum leaf height represents the species' ability to capture light in the community (Westoby 1998). LMA represents an investment of leaf resources in stress tolerance and is strongly correlated with photosynthetic traits such as photosynthetic capacity and leaf nitrogen concentration (Westoby 1998; Hikosaka 2004). Leaf size as related to boundary layer resistance helps to maintain favourable leaf temperatures and is subject to allometric constraints of plant size, shoot size, leaf morphology or size of reproductive organs (Milla and Reich 2011). The maximum leaf height was measured in the field in at least 10 individuals per species per transect and the mean value was calculated. To measure individual leaf area, at least 12 leaves from 6 individuals per species per transect were scanned in the laboratory and measured using Image J, ver. 1.37 software (Wayne Rasband, NIH, Bethesda, MD, USA). After scanning, the leaves were oven-dried at 70 °C for at least 3 days to measure the dry mass, which was then used to determine LMA.

Environmental factors

The pH and electric conductivity (EC; μS/cm) of soil solution in proximity to each quadrat ($n = 810$) were measured by using digital pH and EC meters (B-212 and B-173, respectively, Horiba Ltd., Kyoto, Japan). At each site, we placed a temperature logger (model U23-001, HOBO Pro V2 Temp/RH data logger, Onset Computer Corp., Bourne, MA, USA) in the shade of trees at a height of 120 cm from the ground; air temperature (°C; $n = 27$) was measured every 1 h from mid-August to the end of September in 2009, and values were averaged across this period. To determine the duration of snow cover, we placed a temperature logger (Stow Away TidbiT; Onset Computer Corp.) on the ground surface at each site at the end of the growing season in 2009 and collected them after snowmelt in 2010 ($n = 27$). Altitude and geographic coordinates were measured with a differential GPS receiver (Sokkia GIR1600; Sokkia Co. Ltd., Tokyo, Japan).

Data analysis

Parameterization

We organized the data into a single matrix of 4 environmental factors × 27 sites and subjected the matrix to a principal component analysis (PCA) based on the correlation matrix of variables, in which the data are centred and standardized by standard deviation. The four environmental factors analyzed for each site were altitude, temperature, mean water pH and mean water EC. We did not use the duration of snow cover in this analysis, because we lost some of temperature loggers placed on the ground surface in the field. We used representative environmental values of each site as PCA axes.

We determined the site-mean values of the three leaf traits, referring to Cornwell and Ackerly (2009), for use in the following calculations. The abundance-weighted site-mean trait value was calculated as follows:

$$\bar{t}_j = \frac{\sum_{i=1}^{S} A_{ij} t_{ij}}{\sum_{i=1}^{S} A_{ij}} \quad (1)$$

where t_{ij} is the trait value of species i at site j, A_{ij} is the coverage of species i at site j and S is the number of species at each site. We calculated abundance-weighted species-mean environmental values to represent the degree of species preference for a position in the niche along the environmental gradient by using the scores of PCA axes:

$$\bar{E}_j = \frac{\sum_{i=1}^{P} A_{ij} E_{ij}}{\sum_{i=1}^{P} A_{ij}} \quad (2)$$

where E_{ij} is the PCA axes score of species i at site j and P is the number of sites in the study area. The abundance-weighted species-mean trait values as a measure of the leaf traits of each species, considering intraspecific variation among study sites, are defined in the same way:

$$\overline{t_i} = \frac{\sum_{j=1}^{P} A_{ij} t_{ij}}{\sum_{j=1}^{P} A_{ij}} \tag{3}$$

Species of vascular plants were classified into two leaf habits: evergreen and deciduous; evergreens were defined as species that had green leaves in winter. Species were also classified into two growth forms: shrubs and herbs; shrubs were defined as species that had a wintering stem above the ground. Therefore, species were classified into four FGs: evergreen shrubs, evergreen herbs, deciduous shrubs and deciduous herbs. We did not consider photosynthetic types (i.e. C3 or C4) because all the studied species were considered C3. *Myrica gale* was a nitrogen fixer but we ignored because of the limited number of species. The abundance-weighted FG-mean environmental values were calculated as:

$$\overline{E_g} = \frac{\sum_{j=1}^{P} \sum_{i=1}^{Sg} A_{ij} E_{ij}}{\sum_{j=1}^{P} \sum_{i=1}^{Sg} A_{ij}}, \tag{4}$$

where g is FG and S_g is the number of species belonging to a certain FG. Likewise, the abundance-weighted FG-mean trait values were calculated as:

$$\overline{t_g} = \frac{\sum_{j=1}^{P} \sum_{i=1}^{Sg} A_{ij} t_{ij}}{\sum_{j=1}^{P} \sum_{i=1}^{Sg} A_{ij}}. \tag{5}$$

Contribution of within- and among-species and within- and among-FG variations to the site-level variation along the environmental gradient

The site-level variation of traits along an environmental gradient may be an integration of intraspecific variation, species replacement within a FG and species replacement among FGs. To evaluate each contribution, we calculated scaling slopes (b) with type I regression for the following five relationships: (1) trait value versus PCA score for each species (intraspecific variation),

$$\log t_{ij} = a_{t_{ij}} + b_{t_{ij}} E_{ij}, \tag{6}$$

where a is the intercept. (2) species-mean trait value versus species-mean PCA score across all studied species (among-species variation),

$$\log \overline{t_i} = a_{\overline{t_i}} + b_{\overline{t_i}} \overline{E_i}. \tag{7}$$

(3) species-mean trait value versus species-mean PCA score within each FG (within-FG variation),

$$\log \overline{t_i} = a_{\overline{t_{ig}}} + b_{\overline{t_{ig}}} \overline{E_i} \tag{8}$$

(4) FG-mean trait value versus FG-mean PCA score (among-FG variation),

$$\log \overline{t_g} = a_{\overline{t_g}} + b_{\overline{t_g}} \overline{E_g} \tag{9}$$

(5) Site-mean trait value versus the PCA score (community variation).

$$\log \overline{t_j} = a_{\overline{t_j}} + b_{\overline{t_j}} \overline{E_j} \tag{10}$$

To quantify the relative contribution of intraspecific variation, species replacement, within-FG variation and FG replacement to the community variation, we calculated hypothetical scaling slopes of the community variation, where no variation in trait values within species or within FGs is assumed, as follows:

$$\overline{t'_j} = \frac{\sum_{i=1}^{S} A_{ij} \overline{t_i}}{\sum_{i=1}^{S} A_{ij}} \tag{11}$$

$$\log \overline{t'_j} = a_{\overline{t'_j}} + b_{\overline{t'_j}} \overline{E_j} \tag{12}$$

$$\overline{t''_j} = \frac{\sum_{g=1}^{4} A_{gj} \overline{t_g}}{\sum_{g=1}^{4} A_{gj}} \tag{13}$$

$$\log \overline{t''_j} = a_{\overline{t''_j}} + b_{\overline{t''_j}} \overline{E_j} \tag{14}$$

where A_{gj} is the total coverage of each FG. We then estimated the relative contribution (C) of intraspecific variation, C_i; among-species variation (species replacement), C_I; within FG variation, C_g; and among-FG variation (FG replacement), C_G; to the community slope:

$$C_i = 1 - \frac{b_{\overline{t'_j}}}{b_{\overline{t_j}}} \tag{15}$$

$$C_I = 1 - C_i \tag{16}$$

$$C_g = 1 - \frac{b_{\overline{t''_j}}}{b_{\overline{t_j}}} \tag{17}$$

$$C_G = 1 - C_g. \tag{18}$$

Performance of functional groups in each community

We examined the relationship between species occurrence number per site and the site PCA score in each FG with a simple generalized linear model. In this analysis, we evaluated species number based on vegetation data measured along the six transects at each site. Plants were classified as evergreen or deciduous species and shrubs or herbs, and herbaceous species were further classified into graminoids and forbs.

Results

Patterns of environmental factors

Among the 27 study sites, altitude varied from 574 to 1285 m a.s.l., mean daily temperature varied from 10.69 to 14.19 °C and number of snow-cover days varied from

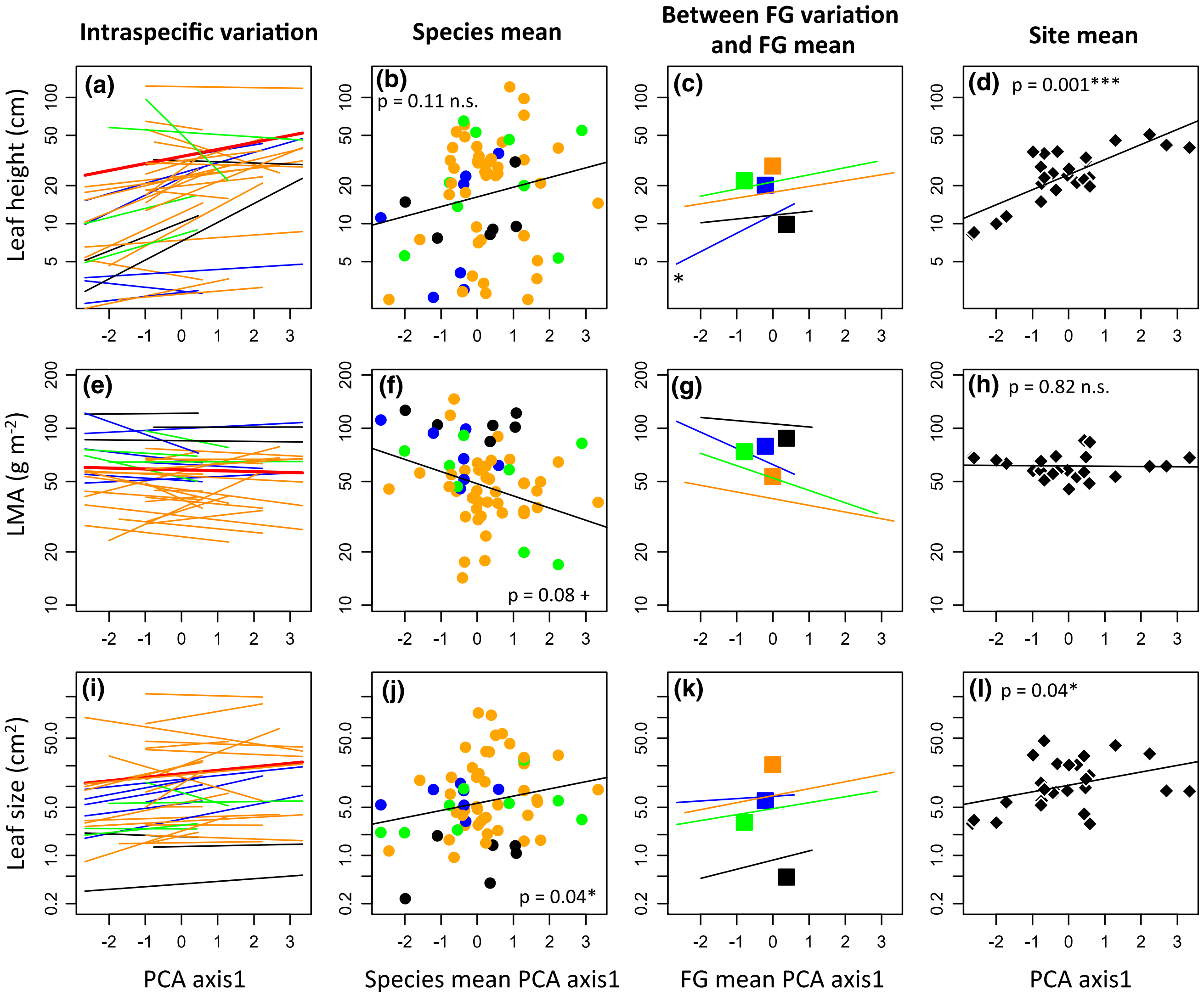

Fig. 1 Relationship between PCA 1 score and maximum leaf height (**a**, **b**, **c**, **d**), LMA (**e**, **f**, **g**, **h**) and leaf size (**i**, **j**, **k**, **l**). Panels (**a**), (**e**) and (**i**) show intraspecific variation in leaf traits in each species, where each line represents the least-squares fit across the PCA 1 score. Panels (**b**), (**f**) and (**j**) show interspecific variation in species-mean leaf traits with species-mean PCA 1, and each line shows the least-squares regression for the species-mean trait. Panels (**c**), (**g**) and (**k**) show within-functional group (FG) and among-FG variation in leaf traits, where *each line* represents the least-squares fit across mean PCA 1 score of the species belonging to a certain FG and *symbols* represent FG-mean PCA 1 and FG-mean traits, respectively. Panels (**d**), (**h**) and (**l**) show site-mean trait value across PCA 1, and *each line* shows the least-squares regression. The significance of regression lines was tested with *t* test ($+P<0.1$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Colours of lines and symbols denote FGs: *orange*, deciduous herbs; *green*, deciduous shrubs; *blue*, evergreen herbs; *black*, evergreen shrubs. *Red lines* in panels (**a**), (**e**) and (**i**) denote the dominant species with the widest range of PCA 1, *Moliniopsis japonica* (deciduous graminoid)

141 to 199 days, respectively (Supplementary Table A1). Mean water pH and EC varied from 4.0 to 5.4 and 45.9 to 221.2 μS/cm.

PCA axis 1 (PCA 1) was identified as representing altitude and temperature and accounted for 53.2 % of the total variance (Table 1). At the lower end of PCA 1 were sites with high altitude and low temperature. PCA axis 2 (PCA 2) appeared to be related to water pH and EC and accounted for another 26.2 % of the total variance. At the lower end of PCA 2 were sites with low pH and low EC.

Community structure

Species level

We recorded a total of 96 species belonging to 42 different families across the entire landscape. Species richness ranged from 13 to 44 species per site, with an average of 29 species. An environmental (PCA) niche width of each species varied from 0.0 (emerging at only one site) to 6.0 on PCA 1 and from 0.0 to 4.0 on PCA 2.

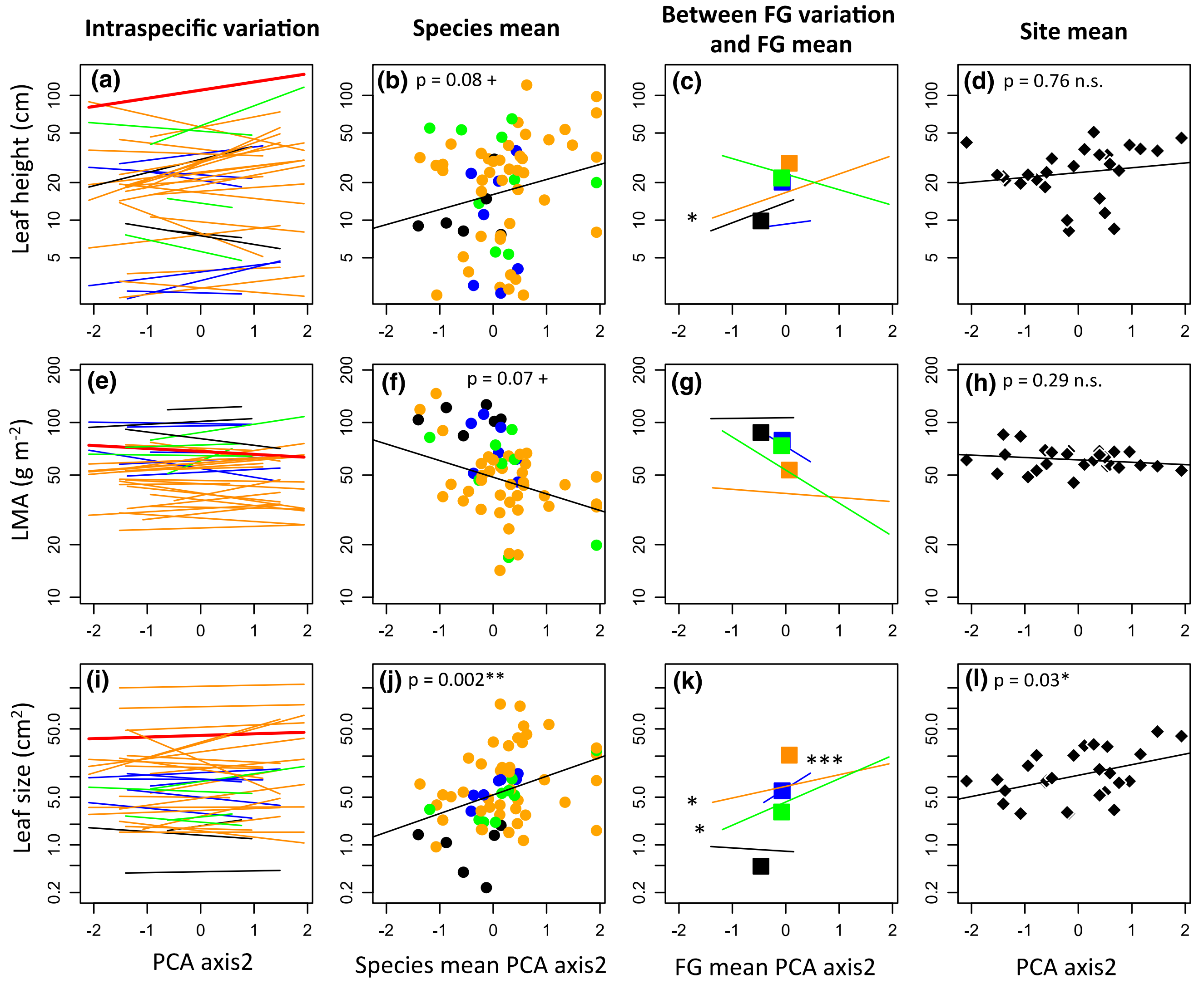

Fig. 2 Relationship between PCA 2 score and maximum leaf height (**a**, **b**, **c**, **d**), LMA (**e**, **f**, **g**, **h**) and leaf size (**i**, **j**, **k**, **l**). Panel descriptions are as described in Fig. 1. *Red lines* in (**a**), (**e**) and (**i**) denote the dominant species with the widest range of PCA 2, *Phragmites australis* (deciduous graminoid)

We measured leaf traits for 72 of the observed species. Maximum leaf height ranged from 2.5 to 151 cm, LMA from 10 to 191 g/m^2 and leaf size from 0.2 to 331 cm^2. To detect intraspecific variation, linear regression was applied to the 36 species that appeared at more than five sites. With respect to PCA 1, 34 of 36 species showed a trend in maximum leaf height (24 positive, 10 negative; Fig. 1a), 29 species showed a trend in LMA (10 positive, 19 negative; Fig. 1e) and 33 species showed a trend in leaf size (25 positive, 8 negative; Fig. 1i). With respect to PCA 2, all 36 species showed a trend in maximum leaf height (21 positive, 15 negative; Fig. 2a), 31 species showed a trend in LMA (20 positive, 11 negative; Fig. 2e) and 33 species showed a trend in leaf size (19 positive, 14 negative; Fig. 2i).

Species-mean values of leaf traits were calculated to assess the interspecies variation. Although most of the species-mean leaf traits were correlated with species-mean values of the PCA axes, the correlations were weak (Figs. 1b, f, j; 2b, f, j). Leaf size increased significantly with increasing PCA values on both axes 1 and 2 (Figs. 1j, 2j). The relationships between LMA and PCA values were positive but only marginally significant ($P < 0.1$; Figs. 1f, 2f). Although species with higher mean of maximum leaf height were absent from the lower end of PCA 1, the relationship between species-mean PCA 1 and species-mean of maximum leaf height was not significant (Fig. 1b). There was a positive, marginally significant relationship between species-mean PCA 2 and species-mean of maximum leaf height (Fig. 2b).

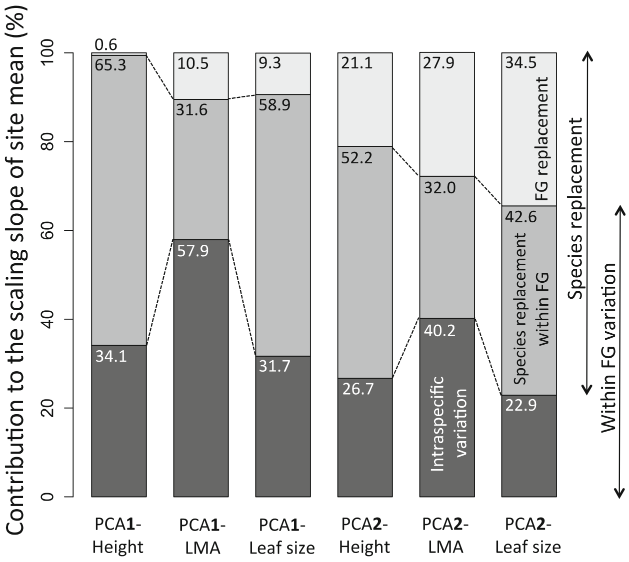

Fig. 3 The scaling slopes of each site-mean trait versus environment relationship attributed to intraspecific variation, species replacement and functional group (FG) replacement, which are expressed as percentages of the inclement from slopes with no species and FG variation considered (see Eqs. 8, 9, 10, 11)

Table 1 Eigenvector scores of plant traits on the two main PCA axes, obtained from a matrix of 4 environmental factors × 27 sites. Water EC is an electric conductivity (μs cm^{-1}) of soil solution

Environmental factor	PCA 1 (53.2 %)	PCA 2 (26.2 %)
Altitude	**–0.654**	0.134
Temperature	**0.624**	–0.217
Water pH	0.418	**0.345**
Water EC	0.087	**0.903**

Values are ranked in order of absolute magnitude along PCA 1. The two highest eigenvector scores for each PCA axis are indicated in bold. Values in parentheses indicate variance accounted for by each axis

Functional group level

Table 2 provides the total emerging species number in each FG and correlations between the number of species in each FG and the eigenvector scores of PCA 1 and PCA 2. The number of evergreen species was significantly correlated with PCA 1, and the number of evergreen herbs showed a marginally significant correlation with PCA 2 (Table 2), which indicates that evergreen species tend to inhabit higher altitudes and sites with lower nutrient availability (Fig. 1b, f, j; Fig. 2b, f, j). The correlation between the species number and PCA 2 was positive for deciduous shrubs, total shrubs and forbs and negative for graminoids and evergreen herbs (Table 2). However, these trends were not observed when the relationships were tested using species for which we measured leaf trait values, except for evergreen herbs.

Some trait values varied among FGs (Supplementary Table A2). At each site, evergreen and herbaceous species had significantly lower maximum leaf heights than those of deciduous and woody species, respectively (Figs. 1a, e, i; 2a, e, i). However, evergreen shrubs had a lower leaf height than deciduous herbs, and only deciduous shrubs had a higher maximum leaf height than herbaceous species. Evergreen and woody species had higher LMAs and smaller leaf sizes than those of deciduous and herbaceous species, respectively, at each site.

To detect within-FG variation, linear regression was applied to each of the four FGs: deciduous herbs, deciduous shrubs, evergreen herbs and evergreen shrubs (Figs. 1c, g, k; 2c, g, k). For maximum leaf height and leaf size with respect to PCA axes, all FGs showed positive trends; these were not significant in most cases, but were consistent with interspecific trends (Figs. 1b, j; 2b, j), except for deciduous shrubs for maximum leaf height versus PCA 2 (Fig. 2c) and evergreen shrubs for leaf size versus PCA 2 (Fig. 2k). All FGs showed negative trends for LMA with respect to PCA axes (Figs. 1g, 2g), which was consistent with interspecific trends (Figs. 1f, 2f), but the within-FG trends were not significant.

We assessed FG-mean values of leaf traits. Relationships between FG-mean traits and the FG-mean PCA 1 score were not significant ($P > 0.1$) (Fig. 1c, f, i). With respect to PCA 2, however, there were significant positive correlations for maximum leaf height and leaf size ($P < 0.001$), indicating a trend in which evergreen shrubs with lower and smaller leaves preferred sites lower on PCA 2 and deciduous herbs with higher and larger leaves preferred sites higher on PCA 2 (Fig. 2c, i). Although a negative correlation was found between FG-mean LMA and FG-mean PCA 2 ranging from evergreen shrubs to deciduous herbs (Fig. 2f), it was not significant ($P > 0.1$).

Community level

Analysis of the relationships between site-mean trait values and PCA scores revealed significant correlations for maximum leaf height and leaf size with PCA 1 and for leaf size with PCA 2 (Figs. 1d, l; 2l). Other relationships were not significant (Figs. 1h; 2d, h), even when species-mean values were significantly correlated.

Relative contribution of traits at the species and FG levels to that at the community level

We calculated scaling slopes of the relationships between trait and PCA values at five levels: within- and among-species, within- and among-FGs and community levels. In all combinations of leaf traits and PCA axes, intraspecific slopes tended to be shallower than the multi-species slopes (among-species, within-FG and among-FG slopes) but similar to the slope of the community level (Fig. A1).

Figure 3 shows the relative contributions of intraspecific variation, species replacement and FG replacement to the community-level slope of the relationship between leaf trait values and PCA scores. Their contributions varied depending on the case. For maximum leaf height versus PCA 1, species replacement within FGs explained 65.3 % of the variation in the community-level slope and intraspecific variation explained most of the rest; FG replacement contributed little to the community-level slope. Species replacement within FGs was also most responsible for leaf size versus PCA 1, whereas intraspecific variation accounted for most of LMA versus PCA 1. This trend was similar to the trait gradient along PCA 2. The contribution of FG replacement was greater along PCA 2 than PCA 1. In particular, for leaf size versus PCA 2, FG replacement made a greater contribution than that of intraspecies variation.

Discussion

Patterns of environmental factors

The environmental variance among our study sites was explained mainly by two independent environmental gradients. The first, PCA 1, represents an altitudinal stress gradient comprised of altitude itself and temperature (Körner 2003, 2007). Although we did not use the duration of snow cover in this analysis, it was significantly correlated with PCA 1 ($n = 18$, $P < 0.01$), indicating that snow cover lasts longer at higher altitudes with lower temperature. The second gradient, PCA 2, represents the moorland water characteristics of water pH and EC. At our study sites, there was no significant correlation between altitude and pH ($P > 0.05$). This finding contrasts with those of Hájková et al. (2006), who found a strong correlation between altitude and pH in the Bulgarian high mountains. Such a relationship may exist because peat accumulates in water with low pH (Proctor and Maltby 1998, Tsuyuzaki et al. 2004), where decomposition is prevented by anaerobic conditions and low temperature (Brady and Weil 1998). At our study sites, the contribution of water flow from adjacent forests differed among the moorland sites depending on microtopography, which might influence water conditions. Thus, our results suggest that topographic features, in addition to temperature and altitude, may affect species composition in moorland communities.

Contribution of intraspecies variation, species replacement and FG replacement to the environmental dependence of trait values at the community level

We noted general trends of environmental dependence of leaf trait values at the within- and among-species levels. Plants inhabiting sites with high altitude and low temperature (low values on PCA 1) and those with low EC and pH water conditions (low values on PCA 2) tended to have lower maximum leaf height, higher LMA and smaller leaf size. Our results are consistent with previous reports on the environmental dependence of maximum leaf height (altitude: Körner et al. 1989, nutrient availability: Westoby 1998), LMA (altitude: Körner et al. 1989; Diemer et al. 1992; Cunningham et al. 1999; Hikosaka et al. 2002, nutrient availability: Cunningham et al. 1999) and leaf size (altitude: Körner 2003; Tang and Ohsawa 1999, nutrient availability: Cunningham et al. 1999).

At the community level, significant correlations were found for maximum leaf height versus PCA 1, leaf size versus PCA 1 and leaf size versus PCA 2 (Figs. 1, 2). The relative contributions of intraspecific variation, species replacement within FGs and FG replacement differed considerably among these relationships (Fig. 3). FG replacement contributed little to the maximum leaf height–PCA 1 relationship but contributed more than 34 % to the leaf size–PCA 2 relationship. Species replacement within FGs made the largest contribution to the three relationships, and intraspecific variation also made a large contribution. These results suggest that the causes of community-level changes in leaf trait values along an environmental gradient vary among cases.

We found that species replacement within FGs made a large contribution to the leaf trait variation at the community level. Previous studies reported that separating species into FGs improves the ability to predict trait values (Reich et al. 2007, Ordoñez et al. 2010), because the values of many plant traits vary significantly among FGs. However, there is great overlap in trait values among FGs (Wright et al. 2004, 2005). Therefore, within-FG variation may play an important role in the variation in community functions.

In this study, intraspecific variation made a significant contribution to the environmental dependence of leaf trait values at the community level (23–58 %). In contrast, in a study of the community response to water availability, the contribution of intraspecific variation was only 14–21 % (Cornwell and Ackerly 2009). Why did intraspecific variation have a relatively strong effect on the community-mean (site-mean) slope in this study? At our study sites, the β diversity among moorlands was high (Sasaki et al. 2012a), but such turnover might occur mainly in rare subordinate species. The environmental (PCA) niche width of each species was positively correlated with species-mean abundance per site (PCA 1: $r = 0.57$, $P < 0.001$; PCA 2: $r = 0.61$, $P < 0.001$), indicating that species with wide environmental niches tended to be abundant at each site. Because community-mean traits were calculated as abundance-weighted values, the community-mean trend along an environmental gradient was greatly affected by intraspecific trait changes in dominant species with a wide distribution rather than by species and FG replacement. The trend in community variation was very similar to that of the most dominant species: *Moliniopsis japonica* (deciduous graminoid) with PCA 1 and *Phragmites australis* (deciduous graminoid) with PCA 2 (shown as red lines in

Table 2 Total emerging species number in each functional group and correlation (*r*) between occurrence number of each functional group at each site and the eigenvector scores of PCA axes 1 and 2 based on analyses of the environmental data set (Table 1)

Functional group	Total number	Correlation between occurrence number (per site) and environmental parameters			
		PCA1		PCA2	
Deciduous herbs	59 (48)	0.18	(0.12)	0.22	(0.20)
Deciduous shrubs	21 (11)	0.24	(0.22)	**0.47****	(0.22)
Evergreen herbs	7 (7)	**–0.68*****	**(–0.68***)**	**–0.25**†	**(–0.25**†**)**
Evergreen shrubs	9 (6)	**–0.51****	(0.10)	0.06	(0.10)
Deciduous total	80 (59)	0.22	(0.10)	0.20	(0.22)
Evergreen total	16 (13)	**–0.67*****	**(–0.61***)**	–0.11	(–0.20)
Shrub total	30 (17)	–0.17	(–0.23)	**0.38****	(0.05)
Herb total	66 (55)	0.02	(–0.05)	0.16	(0.13)
Graminoids	45 (37)	–0.01	(–0.10)	**–0.30**†	(–0.26)
Forbs	21 (18)	–0.03	(–0.02)	**0.33**†	(0.30)

The numbers in parentheses denote the number of species for which leaf traits were measured and the correlation results when only those species were analyzed. Bold values are significant at † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The significance was tested with *t* test. For herbs, species were further classified into graminoids and forbs

Figs. 1, 2, respectively). Such a trend was seen especially in the LMA–PCA 1 and LMA–PCA 2 relationships, where intraspecific variation and abundance nullified the effect of species replacement on the community-level slope (Figs. 1, 2, 3, A1).

Environmental effect on species composition

The degree of dependence of species number on the environmental gradient varied among the FGs (Table 2). A conceptual competition model predicted that conservative species such as evergreens with a slow growth rate and high LMA (Diaz et al. 2004; Wright et al. 2004) will be excluded from more fertile and mild sites by competition with exploitative species with a fast growth rate and low LMA (Grime 1977; Wedin and Tilman 1993). In accordance with this prediction, the number of evergreen species was lower at sites with higher PCA 1 and PCA 2 scores (Table 2). The species-mean value of each evergreen species, which may represent the preferred position in the niche, was also inclined toward sites with more stressful conditions (high altitude with low temperature and low pH and EC; Figs. 1b, f, j; 2b, f, j). Maximum leaf height tended to be lower in evergreen species (Supplementary Table A2), suggesting that evergreen species were shaded by faster-growing or taller species and were at a greater disadvantage in light competition at lower altitudes, where taller deciduous species were more dominant (Kamiyama et al. 2010). However, the lower maximum leaf height of evergreen species may help them to avoid environmental stresses such as wind exposure at high altitudes (Nagano et al. 2009). A long leaf lifespan may also be advantageous at sites with low nutrient availability, because it enhances the mean residence time of nutrients in the plant, thus improving overall nutrient use efficiency (Aerts 1995; Givnish 2002). The number of deciduous shrubs per site increased with the PCA 2 score of the site (Table 2). Having long-lived stems may contribute to maintaining leaves at higher position with smaller costs, which is advantageous for light competition in taller stands (Kamiyama et al. 2010).

Conclusions

The community structure in the moorlands of this study was influenced mainly by two environmental factors: temperature and water condition. Some of the leaf trait values changed significantly along the environmental gradients at the within-species, among-species and community levels. The relative contributions of intraspecific variation, species replacement within FGs and FG replacement to the trend at the community level varied among cases. We found that species replacement within FGs, which has received little attention in previous studies, was the most responsible for community-level changes. We conclude that changes in leaf trait values at the community level may be caused by various mechanisms involving intraspecific variation and species replacement. Understanding such mechanisms should help us better predict how the structure and functioning of moorland plant communities will respond to climate change.

Acknowledgments We thank the lab members for their kindness in helping with the field work, especially Yukari Abe, Sawako Imahiro, Akira Inoue, and Yuta Nihonmatsu. We also thank Koji Yonekura for his advice about species identification and Hiroko Kurokawa for her helpful comments. The study was conducted with permission by the Ministry of the Environment, Aomori Prefecture Government, Aomori City and a landowner, Tashiro farm. This work was carried out under a JSPS research fellowship awarded to CK, and supported by KAKENHI to KH, the Global Environment Research Fund (F-052 and F-092) by the Ministry of the Environment, Japan to KH, a research grant from The Mitsui & Co., Ltd. Environment Fund, and the Global COE Program "Ecosystem Adaptability Science for the Future" (j03) of the MEXT.

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