

Plant functional types and elevated CO₂: A method of scanning for causes of community alteration

U. Grüters*, S. Janze, C. Kammann, H.-J. Jäger

(Received April 28, 2006)

Summary

In this paper, a general method for an *a posteriori* plant functional type (PFT) analysis of global change effects on community composition is developed. We apply the method to a case study, specifically the Giessen-FACE experiment. This experiment involves a Central European meadow that has been exposed to moderate CO₂-enrichment since May 1998.

The method for an *a posteriori* PFT-analysis: The method consists of four working steps and uses a combination of standard gradient analysis and Random Forests (RF). (1) The trait composition of the species is studied using Principal Components Analysis. Species trait information is gathered from databases. Natural PFT, i.e. groups of species with similar trait-sets, are identified specifically for the community under study. (2) A ranking of the species according to standardized/absolute CO₂ abundance response is obtained from Redundancy Analysis. Initially, species with a response above or below the median are grouped into three response groups (RG) each having similar behaviour, i.e. positive/negative or no-response. (3) The outlyingness measure of RF is used to shift RG boundaries until satisfactory RG homogeneity is achieved. RF is utilized to find the best traits for the RG classification. The behaviour of species representative of the RG is derived from RF class centers. (4) From knowledge gained in steps 1-3, hypotheses about the causes underlying the community alteration are built. Strengths/weaknesses of the method are discussed.

Application of the method to the case study: The community consists of three natural PFT. Five species are summer-green forbs of varying competitiveness. Four species are evergreen ruderal forbs characterized as (semi-) basal rosette plants. The third natural PFT contains evergreen, more or less competitive species, mostly grasses, but also a few forbs.

Negative standardized CO₂-response was practically restricted to two natural PFT, i.e. the summer-greens, irrespective of their competitiveness, and the evergreen ruderals. Standard positive response covered part of the evergreen competitive natural PFT. Among them was *Glechoma hederacea*, one of the forbs with the greatest similarity to grasses. Two hypotheses were formulated to explain the response pattern: (1) Summer-greens lost in competition with evergreens, because the annual time-integral they can use for enhanced growth was more limited with year-round CO₂-enrichment. (2) As rosette plants, ruderal evergreens lagged behind evergreen competitors because periods with full sunlight, which enabled them to gain additional carbon, were shorter for them.

Absolute responses were additionally dependent on dominance patterns. The most striking difference to standard responses was the restriction of positive response to (sub-)dominant grasses.

Introduction

A wide range of species-abundance responses to elevated CO₂ has been reported for Free Air CO₂-Enrichment (FACE) experiments

conducted on grassland ecosystems (CAMPBELL et al., 2000; KÖRNER, 2000; NOWAK et al., 2004).

Often, pre-defined or so-called *a priori* plant functional types (PFT) have been used to summarize results of biomass- or abundance-shifts at the species level. The most frequently deployed classification for temperate grassland communities is based on tradition or on taxonomy (grasses and forbs, LÜSCHER et al., 1996; VASSEUR and POTVIN, 1998; EDWARDS et al., 2001; MARISSINK and HANSSON, 2002). This classification encompasses two problems. Firstly, in many experiments no definitive response was detected for these groups, e.g. grasses in Canadian pasture (VASSEUR and POTVIN, 1998), Swedish grassland (MARISSINK and HANSSON, 2002), and C₃ grasses in the short-grass steppe (MORGAN et al., 2004). Secondly, the membership in one of the groups does not comprise *per se* an explanation for contrasting behaviour. As a consequence, the usefulness of this classification has been questioned (TEYSSONNEYRE et al., 2002).

However, the concept of PFT has recently undergone major advancements. In the novel conceptual framework, the first step of PFT analysis is experimental manipulation of a global change factor and the *a posteriori* identification of response groups (RG), each consisting of species with similar behaviour (CHAPIN et al., 2000; CHAPIN et al., 2002; DÍAZ et al., 2002; LAVOREL and GARNIER, 2002; CHAPIN, 2003). In the classification of response groups, species traits play a pivotal role. Distinct species trait-sets are assumed to cause the species to respond differently (LAVOREL and GARNIER, 2002; CHAPIN et al., 2002).

In our opinion, *a posteriori* PFT analysis has the potential to overcome the predicament of elevated CO₂ research summarized by KÖRNER (2000) as „It is possibly one of the greatest frustrations of researchers in recent years that no reliable functional groups with respect to CO₂-responsiveness could be identified“.

The aim of this paper is to develop a general method for an *a posteriori* PFT analysis of global-change effects on plant community composition. The method characterizes the trait composition of the initial plant community, provides a ranking of species responses to the global change factor, identifies response groups and scans for species traits which caused the detected community alteration. It combines standard gradient-analysis techniques and Random Forests (RF) (BREIMAN, 2001), the 21st century advancement to Classification and Regression Trees (CART). The method is applied to an elevated-CO₂ case study, namely the Giessen-FACE experiment – a long-term ecosystem study, in which a Central-European old meadow has been exposed year-round to moderate CO₂-enrichment (20% above ambient level).

1. Description of the Giessen-FACE case study

1.1 Experimental site

The grassland research area is situated on the floodplain of a river near Giessen, Germany (at 50°32'N; 8°41.3'E, 172 m above sea-

level). Due to the floodplain location the soil is fluvic Gleysol with a texture of sandy clay loam over a clay layer (FAO classification). Between 1996 and 2001 the site received a mean annual precipitation of 573 mm and the average annual air temperature amounted to 9.2 °C. For at least 50 years the grassland on this area has been managed as a meadow, mown twice a year. Over the whole time the area was fertilized with nitrogen at a rate of 50 - 80 kg N ha⁻¹ a⁻¹. This management regime was maintained after the establishment of the research station in 1993/1994. However, the nitrogen supply in the years 1993-1995 (80 kg N ha⁻¹ a⁻¹) was reduced to 40 kg N ha⁻¹ a⁻¹ in all years following. Each spring, 600 kg ha⁻¹ a⁻¹ of 10% P₂O₅ + 15% K₂O + 3% MgO; 33% CaO + MgO were supplied to the site. Throughout a pre-experimental phase (1993-1996), soil moisture, composition of the vegetation and above-ground biomass at harvest were monitored. The monitoring revealed that the area was under the influence of a soil moisture gradient. These results enabled to choose locations with equal baseline conditions for the replicate rings.

1.2 Experimental design

In 1997 three FACE ring pairs, resembling Mid-FACE size, were installed along the detected soil moisture gradient (Ambient CO₂ (A) and Elevated CO₂ (E) combined with „dry“ (A1, E1), „intermediate“ (A3, E3) and „wet“ soil conditions (A2, E2)).

The CO₂-enrichment commenced in April 1998. Since then, the enrichment has been in daily operation throughout the entire year from 2 h after sunrise to 2 h before sunset. The exposure system allows for relative increment of ambient CO₂ levels with resembling the diurnal cycle in the ambient. The CO₂ concentration was set at 20% above ambient levels to simulate concentrations expected to be reached within the next 25 - 40 years (HOUGHTON et al., 2001).

The soil moisture was measured once daily with four permanently-installed TDR sensors in each CO₂ ring (Imko, type P2G; vertically

inserted in 0 - 15 cm depth). The four annual means of soil moisture were pooled to give an average for the ring. Further experimental details can be found elsewhere (JÄGER et al., 2003; KAMMANN et al., 2005).

1.3 Sampling the plant community

The vegetation on the research site is an *Arrhenatheretum elatioris* (Br.-Bl.) *Filipendula ulmaria* sub-community (RODWELL et al., 1992). Species richness is moderate on the site and amounts to 16 species on 4.5 m² plots and 35 species on 100 m² plots, respectively. The species-area relation can be described best by the exponential model:

$$\text{Number of Species} = 18.462 \log_{10}(\text{Area}) - 2.3742 \quad (R^2 = 0.9062)$$

where Area denotes the plot size in m².

The dominant species are the grasses *Arrhenatherum elatius* (L.) and *Holcus lanatus* (L.) along with the forb *Galium mollugo* (L.). The abundance of the species in the community was estimated with twice-annual vegetation surveys, preceding each summer (Sum: May 28 until June 16) and autumn harvest (Aut: August 25 until September 11) by one week. The surveys took place on two 4.5 m² permanent plots located in the northern and southern half of each ring. %-coverages for the species were estimated with the Relevé method. The values were pooled to give ring averages (3 replicates of CO₂-treatment). Species having a constancy of less than 10% among plots were excluded from further analysis. Since the original vegetation data held units of %-coverage it had to be logarithmically transformed. By this means a model based on the ecologically more meaningful multiplicative scale could be constructed (LEPŠ and ŠMILAUER, 2003). Multivariate analysis of pre-experimental data revealed no significant differences in species composition between plots selected later for A and E treatments, respectively.

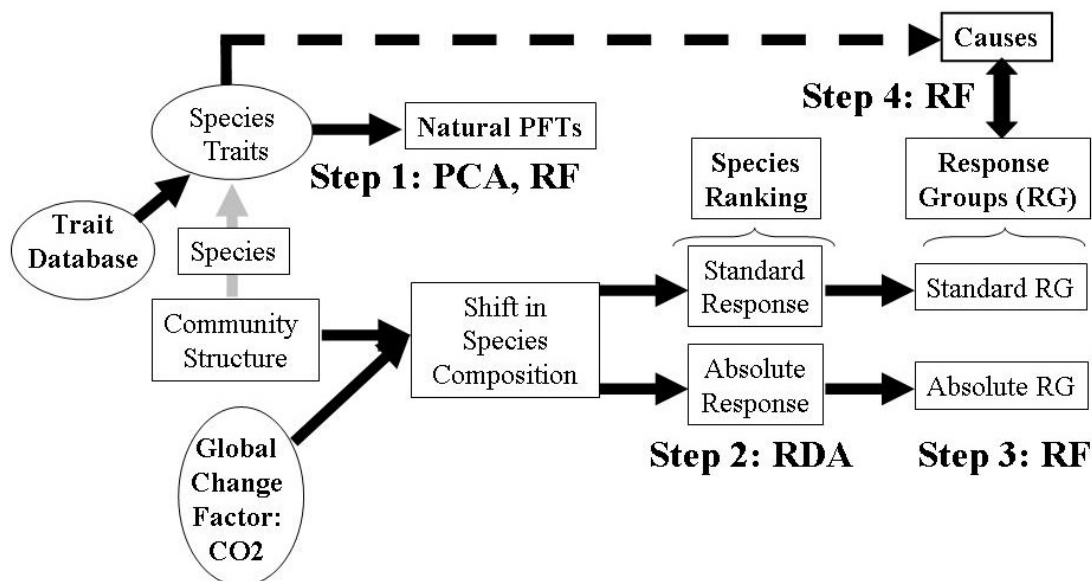


Fig. 1: An outline of the method for an *a posteriori* PFT analysis: For further explanation see text.

Abbreviations: PCA = Principal Components Analysis, RDA = Redundancy Analysis, RF = Random Forests

2. The method for an *a posteriori* PFT analysis

An outline of the method for the *a posteriori* PFT analysis of global change effects is given in Fig. 1. Basically, the method consists of four working steps. Step 1 uses information from species traitbases and a combination of Principal Components Analysis (PCA, TER BRAAK and ŠMILAUER, 1998) and Random Forests (RF, BREIMAN, 2001) to characterize the trait composition of the species and to identify groups of species with similar trait-sets, hereafter called natural PFT. Step 2 provides a ranking of species responses to the global change factor from Partial Redundancy Analysis (RDA, TER BRAAK and ŠMILAUER, 1998). We distinguish two types of ranking. The first is based on standardized species responses to remove effects of varying initial abundance, while the second is obtained from absolute shifts in species abundance. In Steps 3 and 4 the Random Forests algorithm is used to identify homogeneous response groups and to scan for possible causes of community change by an examination of the most important traits for the classification of response groups. The entire gradient analysis (PCA and RDA) was conducted with CANOCO 4.5 (TER BRAAK and ŠMILAUER, 1998). Random Forests were calculated with the similarly-named R-package (LIAW and WIENER, 2006), which is a translation of Breiman's and Cutler's original Fortran code (BREIMAN and CUTLER, 2006) to R. Additionally, in an explorative phase, the partition platform of JMP 5.0.1 (SAS Institute Inc., NC, USA) was used to calculate CART.

The following sections 2.1 - 2.4 describe the working steps in more detail, while the respective sections 3.1 - 3.4 apply the working steps to the case study. In principle, the discussion resembles this scheme by first dealing with the method and then with the application.

2.1 Characterizing the trait composition of the species

A PCA was conducted to examine the trait composition of the species present in the community. The necessary species trait information was gathered from databases (FITTER and PEAT, 1994; HODGSON et al., 1995; KLEYER, 1995). The life history traits included are shown in Tab. 1. In a few instances missing values were supplemented from SCHUBERT and VENT (1990) and OBERDORFER (1990). Leaf nitrogen concentrations were taken from a field survey made in the UK (THOMPSON et al., 1997) and from the GLOPNET database (WRIGHT et al., 2004). With a total of 24 traits, in part categorical and in part numerical variables, a relatively large trait number was incorporated. A core set of six traits was chosen based on the emphasis these traits were given in the CO₂ literature (seedling growth rate, competitiveness, ruderality, stress tolerance, leaf N-concentration, root type), but many more traits previously not known to be influential were also included. Traits that had missing values for more than 8 of the 27 species ($\leq 33\%$) were excluded from the analysis. For the remaining traits, missing values were imputed iteratively from species proximities using Random Forests (see section 2.3). This procedure was not suitable for traits with more than 33% missing values, since the imputation integrates the effects of all other traits and, hence, imputed traits with large numbers of missing values tended to become „super“-predictors in steps 3 and 4.

The linear PCA was chosen, because Detrended Correspondence Analysis (DCA) revealed that trait composition varied only moderately among species. Before conducting the PCA, categorical variables had to be replaced by a set of dummy variables for each category (with 0 = category absent, 1 = category present). PCA results were centered and standardized by traits to remove effects resulting from the different units of the traits. The resulting correlation biplot (Fig. 2) provides a visualization of the relationships of interest. As usual, correlations can be assessed from the ordination diagram using the biplot rule (LEPŠ and ŠMILAUER, 2003). In principle, the ordination

diagram elucidates similarities among the trait composition of the various species and sheds light on relationships among the traits. By this means, it is possible to identify groups of species with similar trait-sets, i.e. natural PFT.

2.2 Species ranking according to standardized/absolute CO₂-response

We distinguish between a species ranking according to standardized CO₂-response and one that is founded on absolute abundance- shifts due to the CO₂-treatment.

The working „sub“-steps for obtaining these two types of species ranking are detailed in the following:

a) DCA: With a gradient length of 1.264 (= average standard deviation of species turnover, TER BRAAK and ŠMILAUER, 1998) the species composition varied only moderately among plots and, hence, the linear method RDA was chosen for direct gradient analysis of the ln-transformed %-coverage data.

b) Redundancy Analysis (RDA) and Empirical Modeling: All explanatory variables considered to affect the community were included in an empirical model (soil moisture, year and season of vegetation survey, CO₂, CO₂ x soil moisture, CO₂ x year, CO₂ x season). The model structure took into account only CO₂-effects beyond existing plot differences. The CO₂-treatment of the year prior to the onset of enrichment (Yr₀ = 1997) was coded as 0 for all plots, though (Before-After-Control-Impact = BACI-Design, TER BRAAK and ŠMILAUER, 1998). This concept particularly allowed for separate statistical testing of CO₂-effects and CO₂ x time interactions. Assuming cyclical behaviour of the species data, experimental time was treated by separating year (Yr₀..Yr₄) and season of the vegetation survey (Sum, Aut), which represents the most general treatment of time (TER BRAAK and ŠMILAUER, 1998). Annual averages of soil moisture were assigned to the data of the corresponding year irrespective of the season in which the vegetation survey was made. Treatment of soil moisture as a variable that changes with the year has important implications: Time effects did not include those of fluctuating soil moisture and CO₂ x soil moisture interactions did not contain effects of CO₂-induced water savings. This was not a problem, since TDR sensor data did indicate that elevated CO₂ induced neither prolonged water savings nor a general shift in soil moisture (KAMMANN et al., 2005).

c) Statistical Testing: The statistical analysis was conducted in the usual way (full model, main effects, interactions, LEPŠ and ŠMILAUER, 2003). Statistical testing was performed by distribution-free Monte-Carlo permutations that were restricted according to the experimental design (only whole-plots freely exchanged). To additionally increase the power of the test, permutations were extended to the split-plot level (alleviated test: cyclic shifts, dependent across whole-plots). This sort of statistical test has also been used by Verdonchot and TER BRAAK (1994) and by TER BRAAK and ŠMILAUER (1998).

d) Partial RDA and ranking of standardized species responses: In the partial RDA the effects of all significant explanatory variables were partialled out as covariables, leaving CO₂ as the only explanatory variable. The species scores along the first ordination axis resulting from the analysis of absolute species composition (samples not standardized by norm, species centered and standardized) reflect standardized CO₂-responses, that are neither dependent on the initial species abundance in the community (LEPŠ and ŠMILAUER, 2003) nor on the transformation of the original data.

Tab. 1: List of generative and vegetative traits used in the *a posteriori* PFT analysis.

Trait	Abbr	Src	Categories	Scale, Var Type
1. Generative Traits				
A. Traits concerning Individual Reproduction				
Time of 1 st Reproduction	TREP	2	6 (of 10) classes, mths, yrs (03,1,2,3,6,ND)	ord, num
Flowering Start	FlowS	1	5 (of 12) classes, month (3,4,5,6,7)	ord, num
Flowering Period	FlowP	1	7 (of 12) classes, months (1,2,3,4,5,8,12)	ord, num
No. of Diaspores/Shoot	NUM	2	3 (of 4) classes, counts (1, 2, ND)	ord, num
B. Traits concerning Mating System/Pollination				
Pollination Type	POL	2	4 (of 6) classes (INS = Insects, WIND = Wind, APO = Apomixis, ND)	nom, cat
C. Traits concerning Dispersal in Space and Time				
Dispersule Weight	DispW	1	4 (of 7) classes, mg, (2,3,4,5)	ord, num
Dispersule Form	DispF	1	3 (of 7) classes (Fr =Fruit, Sd = Seed, ND)	nom, cat
Agency of Dispersal	AD	2	4 (of 4) classes(ANIM = Animals ,WIND = Wind, AQU = Water,UNSP = Unspecific)	nom, cat
Seed Bank Longevity	SeedB	1	4 (of 4) classes (1,2,3,ND)	ord, num
D. Traits concerning Germination and Establishment				
Seasonal Time of Germination	GS	2	7 (of 10) classes (EARLY = Early spring, SPRIN = Spring, EASUM = Early Summer, SUM = Summer, AUT = Autumn, SHED, ND)	nom, cat
Seedling Growth Rate	SGR	3	Species-specific maximum values, g g ⁻¹ d ⁻¹	ord, num
2. Vegetative Traits				
A. Traits concerning Aboveground Vertical Expansion				
Shoot Height	CanH	1	5 (of 10) classes , mm, (1,2,3,4,5)	ord, num
Canopy Structure	CanS	1	3 (of 5) classes (B = Basal rosette, S = Semi-basal rosette, L = Leafy)	nom, cat
Leaf Size	SIZE	2	5 (of 8) classes, cm ² , (1 = LEP = Leptophyllous, 2 = NAN = Nanophyllous, 3 = MIC = Microphyllous, 4 = MES = Mesophyllous, ND)	ord, num
Leaf Phenology	LeafP	1	4 (of 5) classes (Sa = Seasonal aestival, Sh = Seasonal hibernal, Ep = Evergreen partially, Ea = Evergreen always)	nom, cat
Leaf N Concentration	LeafN	5, 6	species-specific values , mg g ⁻¹	ord, num
B. Traits concerning Belowground Expansion				
Root Type	RT	2	7 (of 10) classes (1TUBE = Tuber root, TUSS = Tussock roots, RHIZ = Rhizomes, STOL = Stolons, ELON = Elongated, DEEP = Deep rhizomes, ND)	nom, cat
Rooting Depth	DEEP	2	3 (of 4) classes, m, (0.5, 1, 1.5, ND)	ord, num
Mycorrhiza Type	Myc	1	4 (of 7) classes (VA = vssicular-abuscular, pos = positive, neg = negative, ND)	nom, cat
C. Traits concerning Life History, Life Form, Lateral Expansion, CSR-Status				
Life History	LifeH	1	3 (of 6) classes (As = Summer annual, Aw = Winter annual, P = Perennial)	nom, cat
Life Form	LifeF	1	3 (of 8) classes (Th = Therophytes, Ch = Chamaephytes, H = Hemikryptophytes)	nom, cat
Lateral Spread	LatSp	1	5 (of 6) classes, mm (1,2,3,4,5)	ord, num
Branching	BRA	2	4 (of 5) classes (NO = Without branching, AKRO = Akrotony, BAS = Basitony, ND)	nom, cat
Competitiveness	C-Radius	2, 4	4 (of 5) classes (-2, -1, 0, 1)	ord, num
Stress-Tolerance	S-Radius	2, 4	4 (of 5) classes (-2, -1, 0, 1)	ord, num
Ruderality	R-Radius	2, 4	5 (of 5) classes (-2, -1, 0, 1, 2)	ord, num

Src = Source: 1 = HODGSON et al. (1995), 2 = KLEYER (1995), 3 = FITTER and PEAT (1994), 4 = HODGSON et al. (1999), 5 = THOMPSON et al. (1997), 6 = WRIGHT et al. (2004)

e) Ranking of absolute species responses: To obtain absolute abundance shifts due to CO₂ the following calculations were performed (TER BRAAK and ŠMILAUER, 1998):

$$\text{Spec_Abundance} = \text{Mean_Coverage} + \text{Mean_Coverage} * (\text{EXP}(\text{b_Spec_Ax1} * \text{c_CO2_Ax1} * \text{CO2_std} + \text{b_Spec_Ax2} * \text{c_CO2_Ax2} * \text{CO2_std}) - 1) / 10$$

where Spec_Abandance denotes the %-coverage of either ambient or elevated CO₂-treatment, shifted from the overall mean of the experimental period by only CO₂.

The expression $\text{EXP}(\dots) / 10 (= e^{(\dots) / 10})$ accomplishes back-transformation of the natural logarithm $\ln(10 * Y + 1)$ of the original vegetation data (Y).

b_Spec_Ax1, b_Spec_Ax2 are the non-centered, not standardized scores of one of the species on the first or second ordination axis, respectively. Likewise, c_CO2_Ax1, c_CO2_Ax2 denote the scores of the standardized explanatory variable CO₂ (CO₂_std) on these axes.

The absolute shift in %-coverage due to the CO₂-treatment was calculated by subtracting results for ambient from those for elevated CO₂. Species were ranked accordingly.

2.3 Identification of standardized/absolute CO₂-response groups

In the *a posteriori* PFT analysis proposed here, identification of RG involves finding traits that are shared by group members and that contrast them to species outside the RG. In principle, this represents a classical classification/clustering problem. Nonetheless, traditional unsupervised learning, such as k-means clustering, is not well suited for the problem at hand, because it has difficulties with mixed variable types (DUDA et al., 2001). In contrast, the supervised-learning method CART can easily handle large numbers of both numerical and categorical variables, but requires setting-up RG beforehand from the species ranking.

In an explorative phase, classification trees provided detailed insight into the relationships among species and species traits. Basically, interpretation of the rule sets for RG, which were based on visual inspection and other criteria, was straightforward (DUDA et al., 2001). However, experimentally shifting the class boundaries changed results dramatically. This is a known problem of classification trees and has been referred to as instability (BREIMAN and CUTLER, 2006). Furthermore, CART (in JMP) does not provide a measure for assessing RG homogeneity.

In conclusion, the classifying capabilities of CART appeared to be limited (compare BREIMAN and CUTLER, 2006). The basic problem outlined at the beginning of this section was not solved.

Random Forests (RF) were invented by BREIMAN (2001) to overcome problems known from CART (BREIMAN and CUTLER, 2006). RF builds a population of decision trees for the classification. In the construction of these trees two randomization schemes are used: A boot-strapped sample of cases (i.e. the species) is chosen from the classes (i.e. the RG) using stratified sampling with replacement. These cases are utilized to train the decision tree, whereas the rest of the cases, the „out-of-bag“ cases, form a test dataset and provide a means of calculating an unbiased error-estimate for the tree. At each node the best splitter is determined from 5 randomly-chosen traits. Unlike CART, splitting of a tree in RF is not proceeded until terminal nodes are found for all cases and the tree is eventually subsequently pruned. Instead, splitting is stopped when a terminal node is reached for each class. Thus, RF does not overfit (BREIMAN and CUTLER, 2006) and seems to be even better in this respect than supported vector machines (TRUONG et al., 2004).

The RF classifier works by summing up the votes of the trees for which a case is a test case and dividing by the number of trees. When the RF vote is above a class-specific **cutoff**-threshold the case is given that class. The overall RF error-rate is obtained from the proportion of times a case is misclassified by the RF averaged over all cases. The resulting RF classifier is considered to be unexcelled among current classifying techniques (BREIMAN and CUTLER 2006).

The **importance of variables** (i.e. the traits) is found as the mean decrease in classification accuracy, when the variable in the original dataset is replaced by a variable randomly permuted from the original variable distribution. Additionally, variable importance can be calculated separately for each class. In principle, variable importances focus on the general pattern in the data and represent a sort of change in perspective when compared with the detailed rule sets of CART. From the importance of a variable for the classification one cannot read out the class-specific values of each variable. Towards this end, **class centers** are calculated from the class-specific mode for categorical variables or from the class median for numerical variables (k-nearest neighbours = class members – 1). In essence, class centers give the value of the variable for a typical class member.

However, the most versatile measures in RF are the **species proximities**, which resemble Euclidean distances among the species. They are calculated from the number of times two cases end up in the same terminal node of a tree, summed over all trees and normalized by the number of trees in the forest. Species proximities can be used for **replacement of missing values** (compare section 2.1). They are also useful for detecting **outliers**, defined as cases having low proximity to the other members of their class. They provide a means of solving the problem outlined in the beginning of this section: When a **mislabelled case** at the boundary of a class is detected by its outlyingness the class boundary is shifted. The process is repeated, until RG-homogeneity is satisfactory. Following TRUONG et al. (2004), we considered this to be the case when maximum outlyingness fell below 3. In the alternative case a non-boundary species is an outlier, it might be possible to augment the class size, thereby increasing the „common denominator“ among the species.

Before applying this refinement of class boundaries, however, it is necessary to have an initial set-up of the classes. One might suppose that, using statistical criteria for the separation of species into non-responsive and significantly responding groups is straightforward. However, this is not the case as can be illustrated with the results of the alleviated statistical testing (compare section 3.2): Since the whole set of species was significantly affected by CO₂, any subset consisting of the most responsive species was also significant, but to a greater degree. However, due to the low CO₂-significance of the whole suite of species, removal of only the most responsive species reduced the set to non-significance. Statistical testing of a single species was not possible for most of the species (in CANOCO), possibly because sufficient constancy is required for doing this. Thus, because of the wide range of uncertainty with respect to species significance, almost any grouping could be justified. Due to these difficulties we instead used the simpler approach of LOSOSOVÁ et al. (2004) and divided the set into species responses below (non-responsive) and above the median (positive/negative response).

Special provisions had to be made because RG were imbalanced, i.e. they held different numbers of species. CHEN et al. (2004) advise using the balanced RF scheme of R in the imbalanced case. This is done by down-weighting the bootstrapping sample size(s) of the majority class(es). However, the authors also state that this procedure results in a loss of information, since a large part of the majority class(es) is not used for the construction of the classifier. Due to the low number of species this was not acceptable in our case. Thus,

adjustments were only made to the cutoff-thresholds for the classes to achieve balancing of the classifier.

2.4 Inventory of causes responsible for CO₂-induced community alteration

Step 4 of the method scans for species traits which caused the detected community alteration. To accomplish this task, results from the class centers are interpreted based on knowledge of trait relationships gained in section 2.1. The inventory of causes is solely based on verbal inference, though.

3. Application to the case study

3.1 Characterizing the trait composition of the species

A visualization of the species locations and trait composition among the species is presented in Fig. 2.

With 15.4%, the first ordination axis from PCA, i.e. the horizontal axis, explains most of the variation in the trait composition of the species. It covers an evergreen – summer-green life strategy gradient. Along this axis we find the evergreen strategy on the left and the summer-green strategy on the right. On the vegetative side, summer-greenness is associated with a tuber root, larger leaves, a larger shoot height and a tendency to exhibit higher leaf-nitrogen concentrations. We may ask whether the storage capacity of the tuber root is a prerequisite for high N-concentration and for a summer-green life strategy in general. Among the generative traits, pollination by insects is most prominent. A late time of first generative reproduction might be causally related to summer-greenness, since summer-greens use a shorter time period of the year for active growth. Moreover, summer-greens tend to have heavier dispersules.

Evergreens, on the other hand, are pollinated by wind, but their seeds/fruits are dispersed by animals. Their dispersules appear to stay in the seed bank longer. One of the most prominent vegetative traits is possession of tussock roots. Besides, the evergreens are characterized by a higher seedling growth rate. There is a slight tendency, that these species possess the vesicular-arbuscular mycorrhiza type more often.

The second ordination axis, i.e. the vertical axis, explains slightly less variation in the trait composition than the first. Here, the explained variation amounts to 13.2%. This axis covers a gradient of competitiveness at the bottom towards ruderality at the top. The competitors are mostly defined by a number of vegetative traits: A leafy canopy structure with leaves all along the stem and taller shoots appear to lead to a fast lateral spread. Additionally, they tend to possess mycorrhiza. However, among generative traits, only a delayed flowering start is a characteristic of competitive life strategy. Ruderals, on the other hand, are herbaceous basal or semi-basal rosette plants and consequentially have an evergreen life strategy (to partially avoid competition later in the year). As a consequence, they start flowering early in the year, leading to a long flowering period. Eventually, they can escape the mowing in a flowering state as well, due to the rosette canopy structure. The late time of first generative reproduction might also be causally related to the (semi-)basal canopy structure, although these species, when grown in isolation, tend to have a high seedling growth-rate (SGR). *Plantago* and *Rumex*, in fact, have the second and third-highest SGR ($SGR \geq 0.243 \text{ g g}^{-1} \text{ d}^{-1}$) within the community. As ruderals they produce large numbers of fruits/seeds, which are dispersed by wind. As Fig. 2 shows, ruderals are defined primarily by generative traits.

Looking at the species' location in the ordination diagram, it is most apparent that the grasses cover only a very small region at the

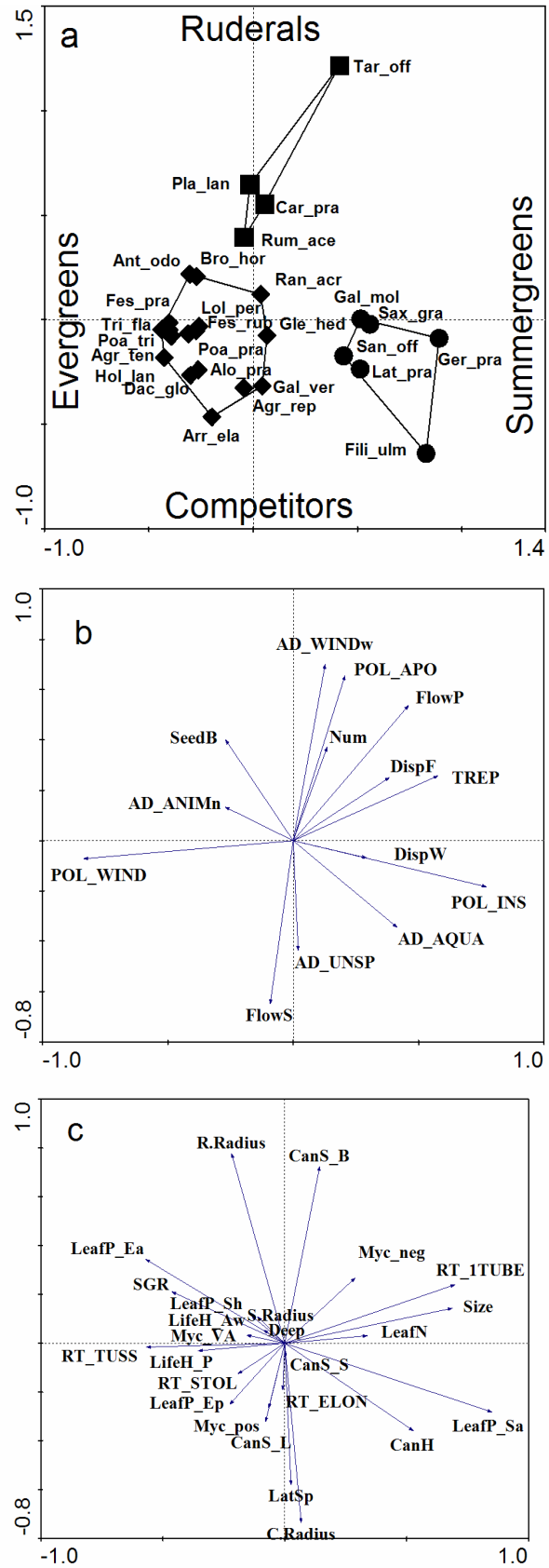


Fig. 2: Ordination diagram of the trait composition for the species obtained from Principal Components Analysis (PCA): a) Species location with envelopes around putative Natural PFT b) Generative traits c) Vegetative traits. For abbreviations used see Tab. 1.

evergreen end of the 1st axis and intermediate along the 2nd, the competitor-ruderal axis. Merely *Arrhenatherum elatius* seems to be slightly more competitive than the other grasses. The trait-sets of grass species are thus very similar to each other. In contrast, herbaceous species possess a much wider spectrum of traits. Most interestingly, some of them even resemble the trait-sets of the grass species. With respect to species CO₂-responses, this implies the following: When the trait-set enables grasses to respond to elevated CO₂ in a certain way, it would not be surprising if the most similar herbaceous species (*Glechoma hederacea*, *Ranunculus acris*, *Galium verum*) responded in the same way. Therefore, we drew an envelope around the grasses and the three forbs and put them in the same natural PFT.

A second natural PFT consists of 4 herbaceous species at the ruderal end of the vertical axis. The third natural PFT contains 5 forbs at the summer-green end of the horizontal axis. Their dissimilarity to the first natural PFT is most pronounced. Among them is likely the most competitive species within this community: The forb *Filipendula ulmaria*.

Some of the trait vectors in Fig. 2b and 2c point towards particular specialists among the species: *Bromus hordeaceus* is a grass that is unique because it is an annual with hibernal leaf phenology. *Taraxacum officinale* is the only species which forms seeds asexually without a requirement for pollination (apomixis). Besides, *Lathyrus pratensis* is the sole legume, but with 48.6 mg g⁻¹ its leaf N concentration is still exceeded by that of *Glechoma hederacea* (52.4 mg g⁻¹).

In conclusion, due to the wide spectrum of traits possessed by herbaceous species in the community under study it would be completely misleading to put them into one natural PFT, named „The Forbs“.

3.2 Species-ranking according to their standardized/absolute CO₂-response

Results obtained from statistical testing of the empirical model are shown in Tab. 2. The design-based test did not show any significant effects of the environmental variables. However, when Monte-Carlo permutations were extended to the split-plot level (alleviated test), the main effects and the effect of moderate CO₂-enrichment were significant. Since none of the interactions was significant, CO₂ appeared to alter the community in a constant manner over the investigation period (1998-2001).

Standardized responses provide the only way to get a true unbiased picture of CO₂-effects on species abundances. As shown in Fig. 3a, the standardized response of the dominant grass *Arrhenatherum elatius*, but also that of the forb *Glechoma hederacea*, was positive and above the median threshold. Hence, both were put in a positive initial response group (RG_Std +). However, the fact that 5 more grasses follow *Glechoma* in the ranking, makes *Glechoma* an outstanding forb. This favourite position represents yet another case in which the *a priori* PFT concept of grasses/forbs would have failed. It is inconsistent with a sole stimulation of grasses, but is consistent with its similarity to grasses. While this supports the validity of the

Tab. 2: Statistical analysis using Monte-Carlo permutation tests: CO₂ = CO₂-treatment, Mois = soil moisture, Yr0 = 1997, year before CO₂-enrichment started, Yr1= 1998, 1st year of CO₂-enrichment,..., Sum = summer records, Aut = autumn records, %-Var. expl. all Canonical Axes = %-variance explained by all canonical axes, P-Value I = p-value of design-based test, P-Value II = p-value of alleviated test.

Null Hypothesis tested	Explanatory Variables	Covariables	% Var. expl. all Canonical Axes	P-Value I P-Value II
H ₀ 1 Full Model	CO ₂ , Mois, Yr0...Yr4, CO ₂ x Mois, CO ₂ x Yr0 ...CO ₂ x Yr4, CO ₂ x Sum, CO ₂ x Aut	Sum, Aut	37.7	0.2860 0.1340
H ₀ 2 Main Effects	CO ₂ , Mois, Yr0...Yr4	Sum, Aut	31.2	0.0550 0.0105
H ₀ 2a	CO ₂	Mois, Yr0...Yr4, Sum, Aut	10.0	0.1550 0.0455
H ₀ 2b	Mois	CO ₂ , Yr0...Yr4, Sum, Aut	8.8	0.1370 0.1155
H ₀ 2c	Yr0...Yr4	CO ₂ , Mois, Sum, Aut	17.2	0.1040 0.0315
H ₀ 3 Interactions	CO ₂ x Mois, CO ₂ x Yr0 ...CO ₂ x Yr4, CO ₂ x Sum, CO ₂ x Aut	CO ₂ , Mois, Yr0...Yr4, Sum, Aut	6.5	0.6100 0.6470
H ₀ 3a	CO ₂ x Mois	CO ₂ , Mois, Yr0...Yr4, Sum, Aut, CO ₂ x Yr0 ...CO ₂ x Yr4, CO ₂ x Sum...CO ₂ x Aut	3.8	0.3870 0.4390
H ₀ 3b	CO ₂ x Yr0 ...CO ₂ x Yr4	CO ₂ , Mois, Yr0...Yr4, Sum, Aut, CO ₂ x Mois, CO ₂ x Sum...CO ₂ x Aut	2.7	0.7160 0.7255
H ₀ 3c	CO ₂ x Sum, CO ₂ x Aut	CO ₂ , Mois, Yr0...Yr4, Sum, Aut, CO ₂ x Mois, CO ₂ x Yr0...CO ₂ x Yr4	1.0	0.9035 0.7555

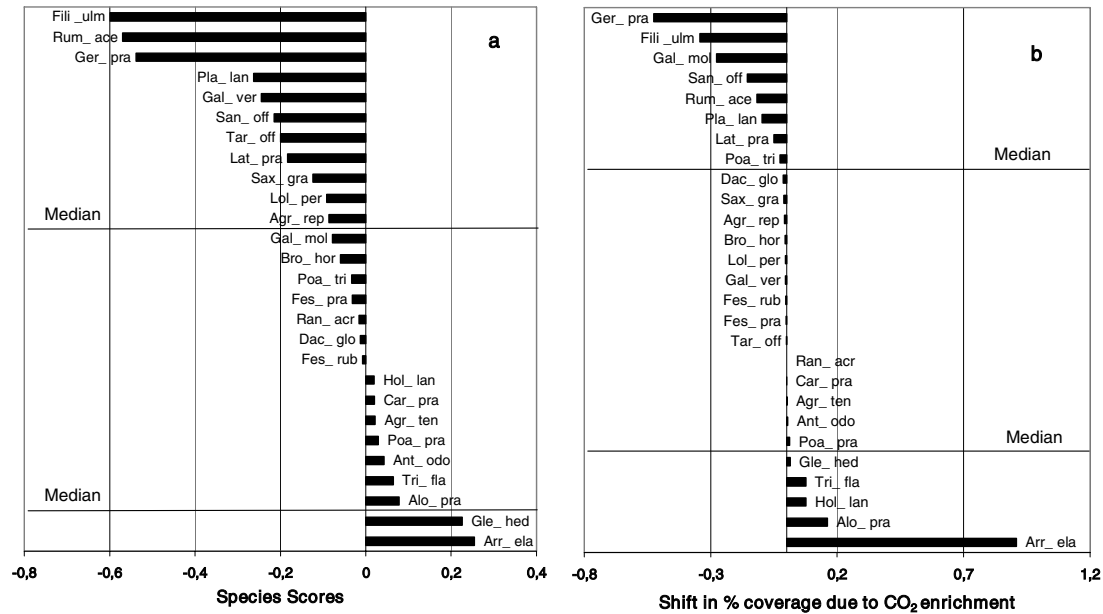


Fig. 3: Ranking of species-specific a) standardized CO₂-responses, b) absolute CO₂-responses. Horizontal lines mark the median threshold.

Abbreviations: **Ger_pra** = *Geranium pratense*, **Fil_ulm** = *Filipendula ulmaria*, **Rum_ace** = *Rumex acetosa*, **Pla_lan** = *Plantago lanceolata*, **San_off** = *Sanguisorba officinalis*, **Lat_pra** = *Lathyrus pratensis*, **Sax_gra** = *Saxifraga granulata*, **Gal_ver** = *Galium verum*, **Lol_per** = *Lolium perenne*, **Agr_rep** = *Agropyron repens*, **Bro_hor** = *Bromus hordeaceus*, **Gal_mol** = *Gallium mollugo*, **Poa_tri** = *Poa trivialis*, **Tar_off** = *Taraxacum officinale*, **Fes_pra** = *Festuca pratensis*, **Dac_glo** = *Dactylis glomerata*, **Ran_acr** = *Ranunculus acris*, **Fes_rub** = *Festuca rubra*, **Car_pra** = *Cardamine pratensis*, **Agr_ten** = *Agrostis tenuis*, **Hol_lan** = *Holcus lanatus*, **Pea_pra** = *Poa pratensis*, **Ant_odo** = *Anthoxanthum odoratum*, **Tri fla** = *Trisetum flavescens*, **Arr_ela** = *Arrhenatherum elatius*, **Alo_pra** = *Alopecurus pratensis*, **Gle_hed** = *Glechoma hederacea*

natural PFT concept, the negative response of *Galium verum* weakens that view.

Overall, nine forbs and only two grasses responded negatively (above the median threshold) and were grouped into an initial negative RG (RG_Std -). The rest of the species, a heterogeneous mixture of grasses (mostly) and forbs, were considered non-responsive by definition (RG_Std 0).

Fig. 3b, at first allows us to assess the absolute response of the whole community. In fact, the reaction of the community as a whole was intrinsically low: All species that responded positively to the CO₂-treatment amount to a total of +1.26 %-coverage, whereas all negative responses summed up to -1.64 %-coverage. This clearly confirms results of the statistical test based on the experimental design.

However, unlike standardized responses, absolute species responses are superimposed by the dominance patterns in the community. Consequently, the two dominant grasses *Arrhenatherum elatius* ($\mu = 39.9$ %-coverage) and *Holcus lanatus* ($\mu = 17.8$ %-coverage) took over the leading position of the positive absolute response. Due to its low abundance *Glechoma hederacea* responded less to elevated CO₂ in absolute terms. From its absolute response *Glechoma* would not have been identified as the outstanding forb. Nonetheless, together with four grasses, this species was still put into RG_Abs +, since its CO₂-induced abundance-shift was larger than the median.

On the negative side, in contrast, *Geranium pratense* and *Filipendula ulmaria* did hold the leading position despite their low abundance, whereas the dominant forb *Galium mollugo* ($\mu = 34.0$ %-coverage) was only third. Basically, with the exception of *Poa trivialis*, the RG_Abs - did consist only of forbs. Once again, in absolute terms a heterogeneous mixture of grasses and forbs was non-responsive (RG_Abs 0).

3.3 Identification of standardized/absolute CO₂-response groups

The adjustment of the class boundaries is carried out in this section. At the end of this process we obtain homogeneous standardized/absolute CO₂-response groups.

Processing the initial standardized RG revealed that the outlyingness of *Galium mollugo* exceeded 5. Therefore, *Galium mollugo* was transferred from RG_Std 0 into RG_Std -. With a total of two species the initial RG_Std + was likely too small for the RF classifier to work under the given constraints: It was not possible to balance the classifier by merely reducing the cutoff-threshold for RG_Std +. Moreover, the outlyingness of *Arrhenatherum* was beyond 30. Following suggestions made in section 2.3, the size of RG_Std + was augmented to reach satisfactory RG homogeneity. The final RG_Std + contained the four most responsive species (see Fig. 3a).

Tab. 3 shows the importance of the traits for the classification and presents the class centers for the final RG, i.e. the trait-sets of a representative RG member. Besides that, Tab. 3 contains the class centers for the natural PFT of evergreen competitors in which RG_Std + was nested.

Overall, the Random Forest misclassified 42.4% of the out-of-bag test cases (Error RG_Std - = 43%, Error RG_Std 0 = 33%, Error RG_Std + = 51.0%). In part, this large error rate was due to the number of traits that did not contribute to the classification and solely represented noise. Additionally, this implies that the species number was at its lower limit for sound RF classification. Nevertheless, when only the five most important traits were incorporated, the error rate improved markedly amounting to 23.8% (Error RG_Std - = 18%, Error RG_Std 0 = 28%, Error RG_Std + = 25%).

In contrast to the standardized RG, the initial absolute RG were kept unchanged. The largest outlyingness among the species amounted to less than 3 (*Poa trivialis*). Tab. 4 shows results concerning variable importance for the classification and class centers for the absolute RG.

Here, the Random Forest misclassified 50.0% of the test cases, when they were out-of-bag (Error RG_Abs - = 47%, Error RG_Abs 0 = 54%, Error RG_Abs + = 49%). Limiting the incorporated traits to the five best classifying ones reduced the overall error rate to acceptable 24.6% (Error RG_Abs - = 13%, Error RG_Abs 0 = 22%, Error RG_Abs + = 39%).

3.4 Inventory of causes responsible for CO₂-induced community alteration

The final step of the *a posteriori* PFT analysis is the inventory of causes responsible for differing RG behaviour.

RF identified the flowering period, the time of first generative reproduction and the root type as the best classifying traits for the standardized RG (Tab. 3).

Species belonging to RG_Std - have the longest flowering period and reproduce themselves late. While the long flowering period is an indicator for summer-greenness and ruderality (see Fig. 2c), the late first generative reproduction and the pollination by insects designate the species as summer-greens. Although at least one clear indicator of summer-greenness (tuber root) was lacking, half of the species were summer-greens and two of them (*Filipendula ulmaria*, *Geranium pratense*) have even decreased in abundance most strongly with CO₂-enrichment (see Fig. 3a). The strong negative response of

the most competitive species (*Filipendula*) suggests that ruderality did not play a major role at the summer-green end. This might explain, at least in part, that clear indicators of ruderality were lacking in the class centers of RG_Std -: The number of dispersules was intermediate and the same was true for the R-radius itself. Nonetheless, three of the four ruderals were members of this group. Species in RG_Std -, thus, largely belonged to two natural PFT: Summer-greens irrespective of their competitiveness and ruderal evergreens.

Species in RG_Std + are primarily flagged as evergreens: Early time of first reproduction was the best classifying trait. The majority of the species is always evergreen and pollinated by wind. Furthermore, the large lateral spread representing the second best classifying trait for the group denotes the species as competitors. This is supported by the leafy canopy structure, the large shoot height and the slightly later flowering start. In line with this is the short flowering period, as it is an indicator for both, degree of evergreen character and competitiveness. However, RG_Std + was nested within the evergreen competitive natural PFT. Comparison of their class centers indicates that rather more competitive species within this natural PFT qualified for positive response. Species in RG_Std + did exhibit larger shoot height, later time of first flowering, shorter flowering period, later flowering start and faster lateral spread than those in the respective natural PFT.

In conclusion, evergreen competitors were the winners in the elevated CO₂-treatment, while summer-greens, irrespective of their competitiveness, and evergreen ruderals lost in abundance. Given that ruderals are (semi-) basal rosette plants, this suggests that overshadowing by neighbours for part of the year caused them to fall behind. But why should the summer-greens lag behind as a group? It has often been reported that *Arrhenathereta* remain green and are

Tab. 3: Variable importance for the classification of standardized RG (decreasing order, in mean decrease of accuracy) and respective class centers including that for the Natural PFT Evergreen Competitors (= Natural PFT EC). Encircled numbers (12345) denote the importance of a variable for the classification of the specific RG. For trait abbreviations used see Tab. 1.

Trait	Mean Decrease Accuracy	RG_Std -		RG_Std 0		Rg_Std +		Natural PFT EC
FlowP	3.27	4	①	3	①	2	③	3
TREP	2.51	2.5	②	1.8	②	1	①	1.74
RT	1.98	RT_ELON	③	RT_TUSS		RT_STOL, TUSS	④	RT_TUSS
Num	1.59	1.26	④	1	⑤	1.5		1
SeedB	1.26	1		2		1	⑤	1
Size	1.24	4		4	④	4		4
LeafP	0.74	LeafP_Ea, Sa		LeafP_Ea		LeafP_Ea		LeafP_Ea
R-Radius	0.59	1.67		2		1.5		2
SGR	0.54	0.171	⑤	0.187		0.191		0.186
LeafN	0.53	29.44		24.3	⑤	26.95		25.49
Myc	0.46	Myc_VA		Myc_VA		Myc_pos, VA		Myc_VA
LatSp	0.46	3		3		3.5	②	3
Pol	0.30	Pol_Ins		Pol_Wind		Pol_Wind		Pol_Wind
FlowS	0.27	5		5		5.5		5
C-Radius	0.12	2		2		2.5		2
CanS	0.12	CanS_S, L		CanS_S		CanS_L		CanS_L
S-Radius	0.11	1.42		2		1.5		2
LifeH	0.00	LifeH_P		LifeH_P		LifeH_P		LifeH_P
DispF	-0.04	Fruit		Fruit		Fruit		Fruit
CanH	-0.07	3		2		3.5		2
AD	-0.27	AD_UNSP		AD_ANIM, UNSP		AD_ANIM, UNSP		AD_UNSP
Deep	-0.50	1		1		1		1
DispW	-0.52	4		3		3		3

able to maintain a positive carbon balance in winter under the mild sub-atlantic climate that prevails in Central-Europe (ELLENBERG, 1996 and citations therein). Thus, it is hypothesized that the more limited annual time-integral they can use for enhanced vegetative growth with year-round CO₂-enrichment caused the competitive loss of summer-greens.

Absolute species responses reflect the dominance patterns within the community as well as the differences in standardized responses. The five best traits for the classification of absolute RG are leaf phenology, time of first flowering, shoot height, root type and seedling growth rate.

Species of RG_Abs – are primarily defined by summer-greenness and related traits (low seedling growth rate, large shoot height, pollination by insects). Unlike RG_Std -, traits associated with ruderality receded in importance for the classification of RG_Abs -. The last remnant of this association is likely the long flowering period exhibited by species of RG_Abs -. The overall low abundance of ruderals in this community is likely to explain this finding.

Early time of first flowering is the best predictor of positive absolute response. As we know from PCA, this trait is primarily associated with the evergreen life strategy. The leaf phenology class center of RG_Abs + confirms this. Two other important classifying traits, namely leafy canopy structure and fast lateral spread, define the respective species as competitors. In principle, this reflects the observation that competitive species tend to be the dominant species as well.

4. Discussion

4.1 The method

To use a metaphor: When compared with the common practice of measuring a few eco-physiological traits on the dominants and relating their response to those traits, knowing the „curriculum vitae“ of all the individual species from a database makes a difference. As visualized in Fig. 2, this knowledge allows to identify natural PFT specific to the community under study. Furthermore, this knowledge makes aware of the (causal) relationships among the traits and provides a background for evaluating the trait-sets of the response groups.

Unlike the *a priori* PFT grasses/forbs, natural PFT were able to give an explanation for the positive response of the forb *Glechoma* among otherwise only grasses, but, unfortunately, could not elucidate why the strikingly similar *Galium verum* responded negatively. Even at closer inspection more indicators for the opposite ranking of the two species were present in the incorporated traits (e.g. *Galium* leafy vs. *Glechoma* semi-basal rosette). Thus, we believe that in the long run only incorporation of more traits could solve this issue.

In working step 1 and throughout the method missing values were a critical issue that could not be solved entirely. Missing values are present in all current databases. However, this situation will presumably change with the LEDA traitbase, since the aim of this EU project was to fill in knowledge gaps and make standardized species traits for the Northwest-European flora available online (KNEVEL et al., 2003).

Tab. 4: Variable importance for the classification of absolute RG (decreasing order, in mean decrease of accuracy) and respective class centers. Encircled numbers (①②③④⑤) denote the importance of a variable for the classification of the specific RG. For trait abbreviations used see Tab. 1.

Trait	Mean Decrease Accuracy	RG_Abs -		RG_Abs 0		RG_Abs +	
LeafP	2.27	LeafP_Sa	①	LeafP_Ea	③	LeafP_Ea	③
TREP	2.25	2.22		1.97	②	1.75	①
CanH	1.68	4	③	2	①	3.5	
RT	1.58	RT_ELON	⑤	RT_ELON, TUSS	⑤	RT_TUSS	④
SGR	1.37	0.166	②	0.184		0.182	
LeafN	0.99	29.44		25	④	40.55	
CanS	0.84	CanS_S		CanS_S		CanS_L	②
FlowP	0.56	4	④	3		2.5	
LatSp	0.55	3		2.5		4.5	⑤
Pol	0.50	Pol_INS		Pol_WIND		Pol_INS, WIND	
DispF	0.38	Fruit		Fruit		Fruit	
Myc	0.33	Myc_pos		Myc_VA		Myc_VA	
Size	0.28	4.27		4		4	
C-Radius	0.17	2.11		2		2.5	
Num	0.11	1		1		1.5	
LifeH	-0.32	LifeH_P		LifeH_P		LifeH_P	
Deep	-0.34	1		1		1	
R-Radius	-0.38	1.69		2		1.5	
SeedB	-0.58	1		2		1.5	
DispW	-0.69	5		3		4	
FlowS	-0.70	5		5		4.5	
AD	-0.73	AD_UNSP		AD_UNSP		AD_UNSP	
S-Radius	-0.75	1.43		1.79		1.5	

The empirical model of working step 2 suffered primarily from the low magnitude of the community response. This likely originated in the applied moderate CO₂-enrichment, but also in the much lower effect size concerning leaf area when compared with aboveground biomass (see KAMMANN et al., 2005). Besides, the power of the statistical tests was low due to statistical noise present in vegetation survey data. Nonetheless, since the ranking of absolute species responses was in good agreement with results of measured biomass for *a priori* PFT (KAMMANN et al., 2005), we proceeded with the *a posteriori* PFT analysis as described.

The ranking of species according to their standardized response was independent of the initial abundances and thus provides an accurate means for comparing responses. The ranking based on absolute abundance shifts, on the other hand, enabled us to assess the magnitude of the community response and to make comparisons with biomass results.

The low species number caused classification problems in working step 3. Thus, the method would be more suitable for experimental scales providing species numbers above the current 27. When species numbers are much larger, as for instance in observational studies, a regression tree approach – as used by DE BELLO et al. (2005) – might be the better choice. However, due to its unexcelled classifying capabilities doing this with Random Forests might still be advantageous.

Overall, the initial set-up of RG and the adjustment of the RG boundaries using the outlyingness measure accomplished RG homogeneity. Results of the RF-classifier were satisfactory when only the best classifying traits were incorporated. However, according to the RF classifier negative standardized CO₂-response was not entirely restricted to the summer-green and the ruderal evergreen natural PFT. In principle, a better congruence with the natural PFT could be obtained by decreasing the outlyingness threshold beyond the value reported in the literature (which was not done here).

In a *posteriori* PFT analysis the trait-set of a response group is assumed to cause the response behaviour. Working step 4 followed this scheme. However, as the cautionary subtitle „A method of scanning for causes...“ suggests, the interpretation of the RG class centers as cause is tentative. We believe that these inferences have more the status of „hypotheses“ and need to be verified either by species-specific eco-physiological measurements or by an organism-based plant community model. In the future, *a posteriori* PFT analysis could benefit a lot from the integration of quantitative methods for causal inference as described by SHIPLEY (2000) and PUGESEK (2002). Causal reasoning could start from basic vegetative traits (e.g. summer-green leaf phenology, basal canopy structure) via a causal chain present in the database, leading to the best classifying traits of RF (flowering period, time of first flowering), and end with the species scores of standardized CO₂-response. In any case, applying Bayesian Networks or Structural Equation Models (SEM) to our case study was not feasible because of the extensive data requirements and because important assumptions of SEM were violated in the data (PETRAITIS et al., 1996).

The method proposed here relies primarily on standard multivariate data-analysis techniques, well-established in ecology. The additional key component of Random Forests is readily available and can be learned with ease. These might represent advantages over the more laborious canonical correlation analysis utilized by LAVOREL et al. (1998).

A large number of species can be incorporated into the PFT analysis as outlined and numerous traits beyond a core set with known significance to the problem can be checked for relevance. This might be beneficial in comparison with the three matrix approach of Pillar

and SOSINSKI (2003) in which an optimal classification is iteratively sought.

In principle, this method may well be applied to investigate the impact of global change factors beyond elevated CO₂ on plant community composition.

4.2 Application to the case study

Similar trait-sets cause species to react in the same way to a global change factor, and natural PFT track these similarities. They do not need to be homogeneous with respect to *a priori* PFT grasses/forbs. Presumably this is one of the most important explanations for the frequent inconsistencies which arose when classification into grasses/forbs was applied.

In essence, negative standardized response to CO₂-enrichment was limited to species belonging to two of the natural PFT, i.e. summer-greens and ruderal evergreens, while the positive standard response covered competitive evergreen species.

POORTER (1993) and POORTER et al. (1996) found fast-growing species (C- and R-type) more responsive to elevated CO₂ than slow-growing ones (S-type). HUNT et al. (1993) screened a total number of 36 individual species for their CO₂-responsiveness and, on the contrary, found competitiveness alone to be the best predictor of the species-specific CO₂-stimulation. Later, POORTER et al. (1996) claimed that Hunt's results also point towards stimulation of fast-growing species (C- and R-type). In principle, it makes sense that an attribute defining the space acquisition capabilities of a species is an excellent predictor of the CO₂-responsiveness of isolated plants (GRIME, 2001). However, a fundamental difference may exist among factors governing species' response in an established ecosystem and those effective on isolated plants as in Hunt's experiments or in non-natural conditions towards which Poorter's meta-analyses were biased. In established ecosystems the response pattern is likely to be modified by biotic factors.

In the meadow under study, the management regime repeatedly frees rosette plants from the competition for light immediately following the first cut in early summer and after the second cut during autumn and early spring of the next year. These time periods appear to enable such ruderals to stay at low abundance in the community under study. It is during these time periods that they receive full sunlight and presumably achieve the largest additional carbon gain with CO₂-enrichment. This is quite distinct from the situation at the forest floor or with artificial, permanent low light, where the CO₂-induced stimulation of growth is most pronounced (HÄTTENSCHWILER, 2001). When light levels are close to the light compensation-point, not enabling positive carbon balance under ambient conditions, CO₂-enrichment will even lead to an infinite relative increment of carbon gain due to the enhanced quantum yield (EHLERINGER and BJÖRKMAN, 1977). Essentially, in this study evergreen ruderals seem to have lagged behind evergreen competitors because of the more limited time-integral with full sunlight available to them. This parallels our hypothesis that summer-green species lost in competition because of the more limited annual time-integral they can use for enhanced vegetative growth with year-round CO₂-enrichment. This argumentation is further supported by the fact that even the most competitive summer-greens decreased in abundance.

However, the possession of a tuber root as part of the inferior „trait syndrome“ of summer-greens was a surprise. It contradicts studies in which storage organs were found to prevent species from establishing a source-sink-imbalance and enable them to retain the initial stimulation of photosynthesis and growth due to CO₂ (POORTER, 1993; POORTER et al., 1996; LÜSCHER et al., 1998; POORTER and NAVAS, 2003). However, we remind the reader that moderate CO₂-enrichment

was applied here and that this exposure regime is less likely to induce a severe source-sink-imbalance than the CO₂-doubling applied in other experiments.

Notably the summer-green *Lathyrus pratensis*, representing the sole legume within the community, also decreased in abundance. In many experimental studies the CO₂-response of leguminous species was more pronounced than that of species without the ability to fix atmospheric nitrogen (POORTER, 1993; LÜSCHER et al., 1996; LÜSCHER et al., 1998; TEYSSONNEYRE et al., 2002). When this was not the case, either an inferior competitiveness for light (TEYSSONNEYRE et al., 2002) or a phosphorous limitation (STÖCKLIN et al., 1998) was held responsible for the finding. Since *Lathyrus pratensis* has considerable canopy height, the first explanation is rather unlikely here. A rough estimation of the annual P-budget of the ecosystem suggests that phosphorus was also not limiting here (see parameters below):

P-supply in fertilizer = 26.2 kg ha⁻¹ a⁻¹

P-withdrawal by harvests = 13.3 kg ha⁻¹ a⁻¹

(assuming average N-withdrawal with harvests = 100 kg N ha⁻¹ a⁻¹,

P:N ratio in harvested plant tissue = 0.133 and

low phosphorus leaching/mobility in the soil)

The sole remaining explanation, however, is that the summer-green trait-set was of higher relevance and that the hypothesized mechanism, however, caused the response of the legume.

In the Giessen-FACE study – as in many other experiments – *a priori* PFT have been studied based on above-ground biomass, which was separated into grasses, forbs and legumes, respectively. A significant increase in above-ground biomass of up to 11% was largely attributable to a stimulation of the *a priori* PFT „grasses“ (KAMMANN et al., 2005). In accordance with this is the ranking of absolute species responses and the finding that positive absolute response was almost restricted to dominant and subdominant grasses. The absence of a total coverage stimulation found here is in line with the unchanged LAI development reported by KAMMANN et al. (2005) for the elevated CO₂-treatment. Both findings indicate that the „taller“ plants had lower leaf area ratio with CO₂-enrichment. The findings further confirm results of the meta-analysis of AINSWORTH and LONG (2005), in which C₃ grasses did show significant biomass increment under FACE conditions, but non-significant LAI-stimulation.

Identification of PFT in a natural ecosystem exposed to elevated CO₂ is a major challenge. Founded on knowledge of the „curriculum vitae“ for all individual species and based on the method for *a posteriori* PFT analysis, it was possible to build several novel hypotheses regarding the causes underlying CO₂-induced community alteration.

Acknowledgements

We gratefully acknowledge financial support from the Hessian Agency for Environment and Geology (Hessisches Landesamt für Umwelt und Geologie, HLUG) and the German Science Foundation (Deutsche Forschungsgemeinschaft, DFG). Petr Šmilauer and Jan Lepš provided valuable advice concerning CANOCO during their course on Multivariate Analysis of Ecological Data. Furthermore, we appreciate the assistance of A. Streitfert and C. Roth, proofreading by G. Richters, and maintenance work of S. Martis, J. Senkbeil, W. Stein, G. Meyer, J. Franz and B. Lenz on the FACE site.

References

AINSWORTH, A.E., LONG, S.P., 2005: What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol.* 165, 351-372.

- BASSIRIRAD, H., CONSTABLE, J.V.H., LUSSENHOP, J., KIMBALL, B.A., NORBY, R.J., OECHEL, W.C., REICH, P.B., SCHLESINGER, W.H., ZITZER, S., SEHTIYA, H.L., LIM, S.I.S., 2003: Widespread foliage δ¹⁵N depletion under elevated CO₂: inferences for the nitrogen cycle. *Glob. Change Biol.* 9, 1582-1590.
- BREIMAN, L., FRIEDMAN, J., STONE, C.J., OLSHEN, R.A., 1984: Classification and Regression Trees. Wadsworth, Redmont.
- BREIMAN, L., 2001: Random Forests. *Mach. Learn.*, 45, 5-32.
- BREIMAN, L., CUTLER, A., 2006: Random Forests. <http://www.stat.berkeley.edu/~breiman/RandomForests/>
- CALDWELL, B.D., HART, A.L., 1996: Competition between grasses and *Trifolium repens* with elevated atmospheric CO₂. In: Bazzaz, F.A., Körner, C. (eds.), Carbon Dioxide, Populations, and Communities, 301-316. Academic Press, San Diego.
- CAMPBELL, B.D., STAFFORD SMITH, D.M., ASH, A.J., FUHRER, J., GIFFORD, R.M., HIERNAX, P., HOWDEN, S.M., JONES, M.B., LUDWIG, J.A., MANDERSCHIED, R., MORGAN, J.A., NEWTON, P.C.D., NÖSBERGER, J., OWENBY, C.E., SOUSSANA, J.F., TUBA, Z., ZUOZHONG, C., 2000: A synthesis of recent global change research on pasture and rangeland production: reduced uncertainties and their management implications. *Agric. Ecosys. Env.* 82, 39-55.
- CHAPIN, F.S. III., ZAVALA, E.S., EVINER, V.T., NAYLOR, R.L., VITOUSEK, P.M., REYNOLDS, H.L., HOOPER, D.U., LAVOREL, S., SALA, O.E., HOBBIE, S.E., MACK, M.C., DÍAZ, S., 2000: Consequences of changing biodiversity. *Nature* 405, 234-242.
- CHAPIN, F.S. III., MATSON, P.A., MOONEY, H.A., 2002: Principles of Terrestrial Ecosystem Ecology. Springer Verlag, New York.
- CHAPIN, F.S. III., 2003: Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. *Ann. Bot.* 91, 455-463.
- CHEN, C., LIAW, A., BREIMAN, L., 2004: Using Random Forest to Learn Imbalanced Data. Technical Report, No. 666, Department of Statistics, University of Berkeley.
- DE BELLO, F., LEPS, J., SEBASTIÁ, M.-T., 2005: Predictive value of plant traits to grazing along a climatic gradient in the Mediterranean. *J. Appl. Ecol.* 42, 824-833.
- DÍAZ, S., BRISKE, D.D., MCINTYRE, S., 2002: Range management and plant functional types. In: Grice, A.C., Hodgkinson, K.C. (eds.), Global Rangelands: Progress and Prospects, 81-100. CABI Publishing, New York.
- DUDA, R.O., HART, P.E., STORK, D.G., 2001: Pattern Classification. John Wiley & Sons Inc., New York.
- EDWARDS, G.R., CLARK, H., NEWTON, P.C.D., 2001: The effects of elevated CO₂ on seed production and seedling recruitment in a sheep-grazed pasture. *Oecologia* 127, 383-394.
- EHLERINGER, J., BJÖRKMAN, O., 1977: Quantum yields for CO₂ uptake in C-3 and C-4 plants - dependence on temperature, CO₂, and O₂ concentration. *Plant Physiol.* 59, 86-90.
- ELLENBERG, H., 1996: Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht. Ulmer Verlag, Stuttgart.
- FITTER, A.H., PEAT, H.J., 1994: The Ecological Flora Database. *J. Ecol.* 82, 415-425. (realized online by H. Ford at <http://www.york.ac.uk/res/ecoflora/cfm/ecofl/index.cfm>)
- GRIME, J.P., 2001: Plant Strategies, Vegetation Processes, and Ecosystem Properties. John Wiley & Sons Inc., New York.
- HATTENSWILER, S., 2001: Tree seedling growth in natural deep shade: functional traits related to interspecific variation in response to elevated CO₂. *Oecologia* 129, 31-42.
- HODGSON, J.G., GRIME, J.P., HUNT, R., THOMPSON, K., 1995: The Electronic Comparative Plant Ecology. Chapman & Hall, London.
- HODGSON, J.G., WILSON, P.J., HUNT, R., GRIME, J.P., THOMPSON, K., 1999: Allocating C-S-R plant functional types: a soft approach to a hard problem. *Oikos* 85, 282-294. (downloadable Excel file to allocate C-S-R types in a graminoid subject, http://www.shef.ac.uk/~nuocpe/ucpe/method_a.xls)
- HOUGHTON, J.T., DING, Y., GRIGGS, D.J., NOGUER, M., VAN DER LINDEN, P.J.,

- DAI, X., MASKELL, K., JOHNSON, C.A., 2001: Climate Change 2001: Working Group I: The Scientific Basis. Cambridge University Press, Cambridge, UK.
- HUNT, R., HAND, D.W., HANNAH, M.A., NEAL, A.M., 1993: Further response to CO₂ enrichment in British herbaceous species. *Funct. Ecol.* 7, 661-668.
- JÄGER, H.-J., SCHMIDT, S.W., KAMMANN, C., GRÜNHAGE, L., MÜLLER, C., HANEWALD, K., 2003: The University of Giessen free-air carbon dioxide enrichment study: description of the experimental site and of a new enrichment system. *J. Appl. Bot.* 77, 117-127.
- KAMMANN, C., GRÜNHAGE, L., GRÜTERS, U., JANZE, S., JÄGER, H.-J., 2005: Response of aboveground grassland biomass and soil moisture to moderate long-term CO₂ enrichment. *Basic Appl. Ecol.* 6, 351-365.
- KLEYER, M., 1995: Biological Traits of Vascular Plants: A Database. Arbeitsberichte Institut für Landschaftsplanung und Ökologie, N.F. Bd. 2, Universität Stuttgart, Stuttgart. (online at <http://www.uni-oldenburg.de/landeco/Download/Databases/biot.htm>)
- KNEVEL, I.C., BEKKER, R.M., BAKKER, J.P., KLEYER, M.A., 2003: Life-history traits of the Northwest European flora: The LEDA database. *J. Veg. Sci.* 14, 611-614.
- KÖRNER, C., 2000: Biosphere response to CO₂ enrichment. *Ecol. Appl.* 10, 1590-1619.
- LAVOREL, S., TOUZARD, B., LEBRETON, J.-D., CLEMENT, B., 1998: Identifying functional groups for response to disturbance in an abandoned pasture. *Acta Oecol.* 19, 227-241.
- LAVOREL, S., GARNIER, E., 2002: Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the holy grail. *Funct. Ecol.* 16, 545-556.
- LEPŠ, J., ŠMILAUER, P., 2003: Multivariate Analysis of Ecological Data using CANOCO. Cambridge University Press, Cambridge, UK.
- LIAW, A., WIENER, M., 2006: Documentation for package 'randomForest' version 4.5-16. <http://www.maths.lth.se/help/R/R/library/randomForest/html/00Index.html>
- LOSOSOVÁ, Z., CHYTRÝ, M., CIMALOVÁ, S., KROPÁČ, Z., OTÝPKOVÁ, Z., PYŠEK, P., TICHÝ, L., 2004: Weed vegetation of arable land in Central Europe: Gradients of diversity and species composition. *J. Veg. Sci.* 15, 415-422.
- LÜSCHER, A., HEBEISEN, T., ZANETTI, S., HARTWIG, U.A., BLUM, H., HENDREY, G.R., NÖSBERGER, J., 1996: Differences between legumes and non-legumes of permanent grassland in their responses to free-air carbon dioxide enrichment: its effect on composition in a multispecies mixture. In: Bazzaz, F.A., Körner, C. (eds.), Carbon Dioxide, Populations, and Communities, 286-299. Academic Press, San Diego.
- LÜSCHER, A., HENDREY, G.R., NÖSBERGER, J., 1998: Long-term responsiveness to free air CO₂ enrichment of functional types, species and genotypes of plants from fertile permanent grassland. *Oecologia* 113, 37-45.
- MARISSINK, M., HANSSON, M., 2002: Floristic composition of a Swedish semi-natural grassland during six years of elevated atmospheric CO₂. *J. Veg. Sci.* 13, 733-747.
- MORGAN, J.A., PATAKI, D.E., KÖRNER, C., CLARK, H., DEL GROSSO, S.J., GRÜNZWEIG, J.M., KNAPP, A.K., MOSIER, A.R., NEWTON, P.C.D., NIKLAUS, P.A., NIPPERT, J.B., NOWAK, R.S., PARTON, W.J., POLLEY, H.W., SHAW, M.R., 2004: Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia* 140, 11-25.
- NOWAK, R.S., ELLSWORTH, D.S., SMITH, S.D., 2004: Functional responses of plants to elevated atmospheric CO₂ – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytol.* 162, 253-290.
- OBERDORFER, E., 1990: Pflanzensoziologische Exkursionsflora. Verlag Eugen Ulmer, Stuttgart.
- PETRAITIS, P.S., DUNHAM, A.E., NIEWIAROWSKI, P.H., 1996: Inferring multiple causality: the limitations of path analysis. *Ecol.* 10, 421-431.
- PILLAR, V., DEPATTA, S., SOSINSKI, J.R., 2003: An improved method of searching plant functional types by numerical analysis. *J. Veg. Sci.* 14, 323-332.
- POORTER, H., 1993: Interspecific variation in the growth response to an elevated ambient CO₂ concentration. *Vegetatio* 104/105, 77-97.
- POORTER, H., ROUMET, C., CAMPBELL, B.D., 1996: Interspecific variation in the growth response to elevated CO₂: A search for functional types. In: Bazzaz, F.A., Körner, C. (eds.), Carbon Dioxide, Populations, and Communities, 375-412. Academic Press, San Diego.
- POORTER, H., NAVAS, M.-L., 2003: Plant growth and competition at elevated CO₂: on winners, losers and functional groups. *New Phytol.* 157, 175-198.
- PUGESEK, B.H., TOMER, A., VON EYE, A., 2002: Structural Equation Modeling: Applications in Ecological and Evolutionary Biology. Cambridge University Press, Cambridge, UK.
- RODWELL, J.S., PIGOTT, C.D., RATCLIFFE, D.A., MALLOCH, A.J., BIRKS, H.J., PROCTOR, M.C., SHIMWELL, D.W., HUNTLEY, J.P., RADFORD, E., WIGGINTON, M.J., WILKINS, P., 1992: British Plant Communities. Volume 3: Grasslands and Montane Communities. Cambridge University Press, Cambridge, UK.
- ROIGER, R.J., GEATZ, M.W., 2003: Data Mining: A Tutorial-Based Primer. Addison Wesley, Boston.
- SCHUBERT, R., VENT, W., 1990: Exkursionsflora von Deutschland, Band 4, Kritischer Band (begründet von Rothmaler, W.). Volk und Wissen Verlag, Berlin.
- SHIPLEY, B., 2004: Cause and Correlation in Biology. Cambridge University Press, Cambridge, UK.
- STÖCKLIN, J., SCHWEIZER, K., KÖRNER, C., 1998: Effects of elevated CO₂ and phosphorus addition on productivity and community composition of intact monoliths from calcareous grassland. *Oecologia* 116, 50-56.
- TER BRAAK, C.J.F., ŠMILAUER, P., 1998: CANOCO Reference Manual and User's Guide to CANOCO for WINDOWS: Software for Canonical Community Ordination (version 4). Microcomputer Power, Ithaca.
- TEYSSONNEYRE, F., PICON-COCHARD, C., FALCIMAGNE, R., SOUSSANA, J.-F., 2002: Effects of elevated CO₂ and cutting frequency on plant community structure in a temperate grassland. *Glob. Change Biol.* 8, 1034-1046.
- THOMPSON, K., PARKINSON, J.A., BAND, S.T., SPENCER, R.E., 1997: A comparative study of leaf nutrient concentrations in a regional herbaceous flora. *New Phytol.* 136, 679.
- TRUONG, Y., XIAODONG, L., BEECHER C., 2004: Learning a complex metabolic dataset using random forests and support vector machines. In: Kim, W., Kohavi, R., Gehrke, J., DuMouchel, W. (eds.), Proceedings of the Tenth ACM SIGKDD International Conference on Knowledge Discovery and Data Mining, 835-840. Seattle, Washington, USA.
- VERDONCHOT, P.F.M., TER BRAAK, C.J.F., 1994: An experimental manipulation of oligochaete communities in mesocosms treated with chlorpyrifos or nutrient additions: multivariate analyses with Monte Carlo permutation tests. *Hydrobiologia* 278, 251-266.
- VASSEUR, L., POTVIN, C., 1998: Natural pasture community response to enriched CO₂ atmosphere. *Plant Ecol.* 135, 31-41.
- WRIGHT, I.J., REICH, P.B., WESTOBY, M., ACKERLY, D.D., BARUCH, Z., BONGERS, F., CAVENDER-BARES, J., CHAPIN, T., CORNELISSEN, J.H.C., DIEMER, M., FLEXAS, J., GARNIER, E., GROOM, P.K., GULIAS, J., HIKOSAKA, K., LAMONT, B.B., LEE, T., LEE, W., LUSK, C., MIDGLEY, J.J., NAVAS, M.-L., NIINEMETS, U., OLEKSYN, J., OSADA, N., POORTER, H., POOT, P., PRIOR, L., PYANKOV, V.I., ROUMET, C., THOMAS, S.C., TJOELKER, M.G., VENEKLAAS, E.J., VILLAR, R., 2004: The worldwide leaf economics spectrum. *Nature* 428, 821-827.

Address of the authors:

Institut für Pflanzenökologie, Justus-Liebig-Universität Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany

* Corresponding author: Dr. U. Grüters

e-mail: Uwe.Grueters@bot2.bio.uni-giessen.de