Assessing functional diversity in the field – methodology matters!

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Summary

1. Interpreting the functional diversity of vegetation is important in unravelling the relationship between environmental change, community composition and ecosystem processes. Functional diversity is the range and distribution of functional trait values in a community. It can be described, among other indicators, by community-level weighted means of trait values (CWM) and functional divergence. Standard methods exist for trait measurements but not for assessments of CWM and functional divergence in the field. No research has addressed the effects of different methods of estimating relative abundances, nor the need to estimate traits at individual, population or species level, or whether methods could be used that bypass taxonomy all together.

2. This study reviews and evaluates plot-level assessment methods of functional diversity in herbaceous vegetation. We asked: (i) Should the objective of the study influence the method for estimating relative abundance? (ii) What are the strengths and limitations of intensive vs. ‘rapid’ approaches, and when should either be applied? (iii) Are taxon-free methods robust in comparison to taxon-explicit methods of trait measurement? Under what circumstances might they be applied?

3. Our review of published studies that have measured functional diversity in the field showed that the choice of metric has not generally taken into account the link between the metric and the functions of interest, and that vegetation cover has been most widely used, regardless of study purpose.

4. We compared quantitatively in subalpine grasslands three methods for quantification of species abundances plus one taxon-free method. We found that: (i) data base trait values were robust across years for a diverse set of dominant species; (ii) CWM have little sensitivity to method for estimating relative abundances; this sensitivity also depends on traits, for example, seed mass results were less stable than leaf traits and heights; (iii) robust estimates of CWM were obtained from visual estimates of species ranks and biomass using a dry-weight ranking method (BOTANAL), whereas functional divergence was more sensitive to method; and (iv) the taxon-free method should be treated with more caution and performed particularly poorly for estimates of functional divergence.

5. We conclude that methodology can affect estimates of functional diversity. Although care should be taken in the choice of method and interpretation of results, rapid methods often offer promising avenues for sampling larger areas and/or repeated measures.

Key-words: community structure, plant functional diversity, methodology, rapid assessments, subalpine grassland
Introduction

Interpreting the functional diversity of vegetation is important in unravelling the relationship between environmental change, community composition and ecosystem processes (Hooper et al. 2005; Grime 2006). In its broadest sense, functional diversity can be defined as the distribution of trait values in a community (Díaz & Cabido 2001; Tilman 2001). Functional diversity can be quantified using a variety of indices that capture different aspects of the distribution of trait values within a community (see recent reviews by Bótta-Dukát 2005; Ricotta 2005; Petchey & Gaston 2006). Research to date has focussed on their theoretical development, linking indices to ecosystem and community processes. In contrast, there has been little focus on the sensitivity of the indices to field measurement methods.

In this paper, we focus on two indices of functional diversity. First, functional divergence quantifies the heterogeneity of trait values within the community by representing the probability that two species picked at random have the same trait value. It is of special interest, as it is considered to reflect niche complementarity (Mason et al. 2005; Petchey & Gaston 2006), but has been little tested in the field (de Bello, Lepš & Sebastià 2006). The second index is the widely used community-weighted mean of a trait (CWM hereafter), defined as the mean of values present in the community weighted by the relative abundance of taxa bearing each value. CWM traits have been shown to be useful to assess community dynamics and ecosystem properties (Garnier et al. 2004, 2007; de Bello, Lepš & Sebastià 2005; Louault et al. 2005; Quétier, Thébault & Lavorel 2007).

The biomass ratio hypothesis (Grime 1998) suggests that it is sufficient to take into account the dominant species from a community to capture response to environmental change and effects on key carbon and nutrient cycling processes (Garnier et al. 2004; Pakeman & Quested 2007). Garnier et al. (2004) showed that CWM for the two most dominant species of Mediterranean old fields were sufficient to capture effects on ecosystem properties relating to the carbon and nitrogen cycles. However, the sensitivity of CWM and functional divergence estimates to different measures or patterns of species abundance, or to variation of trait estimates across methods, has not been investigated.

Measurement of both CWM and functional divergence requires trait measurements of taxa and information on their relative abundance. Standard methods exist to characterize traits (Cornelissen et al. 2003), but little attention has been paid to the method of abundance estimation in relation to these indices. There are a number of well-established methods to assess abundance, and their relative merits have been extensively debated. What has not been examined is how the choice of method can best address the aims of functional diversity studies. When resources for field measurements and data processing are limited, this question is of particular relevance.

Trait measurements can be time consuming for species-rich communities, and require skilled operators. Typically, in herbaceous communities from temperate or Mediterranean regions, it is necessary to measure traits for 5–20 species per community to reach the 80% cumulated abundance which is thought to capture the effects of key ecosystem processes (Garnier et al. 2004). Abundance measurements are similarly demanding on resources, requiring time-consuming methods and botanical expertise. Resource limitation and/or the desire to sample a greater area, or repeat measurements through time, motivate the search for parsimonious (‘rapid’) approaches.

Rapidity can be achieved in two ways: simplifying the method of estimating species abundances and/or streamlining the measurements of plant traits, including ignoring inter-population variation within species, use of trait data bases or foregoing taxonomic identification. This paper reviews and evaluates field assessment methods for herbaceous vegetation in the context of limited time and labour for measurements. We assess the strengths and limitations of different methods of quantifying functional diversity and their applicability to various research questions. We were interested in two components of study objectives: the ecosystem function of interest and the spatial extent of the study. Specifically we asked:

1. Should the objective of the study influence the method of estimating relative abundance?
2. What are the strengths and limitations of ‘intensive’ vs. ‘rapid’ approaches, and when should either be applied?
3. Are taxon-free methods of trait measurement robust in comparison to the taxon-explicit methods that require species identification? Under what circumstances might they be applied?

First, we provide an overview of the available methods and how they have been used in recent literature to estimate CWM and/or functional divergence. We then use a case study of a set of 12 subalpine grassland plots to quantitatively compare both intensive and rapid methods for abundance and/or trait measurements. Drawing from these, we identify a set of key points we believe need to be considered in field studies of functional diversity.

Overview of methods used to assess functional diversity

TRAIT MEASUREMENTS

Two approaches have been used to measure plant traits: taxon-explicit methods and recently developed taxon-free methods that bypass species identification and focus on traits of individual plants. Both approaches can be applied as either an intensive or rapid method, and trade-offs are associated with each. These are summarized in Table 1 and discussed in the following sections.

Taxon-explicit methods

Taxon-explicit methods are needed to examine trait patterns at the species or other taxonomic level. They are also required for traits
relating to population processes (e.g. demographic attributes). The approach is resource intensive in the sense of requiring plant identification skills and the time to differentiate individual plants to the species level.

Standardized methods have been agreed upon for commonly used traits and require trait values to be estimated for c. 20–30 individuals per species or population (Cornelissen et al. 2003). As traits in the same species can vary between sites, sampling populations of the same species across sites has also been advocated (Diaz et al. 1999b). This more intensive method (Table 1) is recommended if genetic variability or trait plasticity is an issue. While some traits respond strongly to environmental variation (e.g. plant height or leaf nutrient concentrations), for others, intraspecific variation in response to site conditions is negligible as compared to interspecific variation (e.g. life history, specific leaf area or seed mass; Garnier et al. 2007; but see Ackerly & Cornwell 2007). Then sampling individuals over the range of sites is the recommended rapid method (Cornelissen et al. 2003).

A second rapid method is to forego field measurements and use pre-existing trait data bases (Grime 1988; Fitter & Peat 1994; Knevel et al. 2003; Gachet, Véla & Tatoni 2005). For a data base to be appropriate for a particular study, it is necessary that trait values be robust to seasonal and interannual variability, although absolute values may not be conserved (Garnier et al. 2001; Roche, Diaz-Burlinson & Gachet 2004). In most cases, data bases provide a single set of trait data that is applied to all the study sites, and their applicability is therefore restricted to questions of interspecific variability. An operational rule for the use of a data base is that the researcher should confirm congruence between interspecies rankings of trait values at the study site and in the data base.

**Taxon-free methods**

Taxon-free methods do not require differentiation of individual plants to the species level and therefore could be of use in high-diversity vegetation. They can also avoid sampling errors between observers or over time (see Ramsay et al. 2006) at sites where species are difficult to differentiate. These methods provide a direct method of assessing functional divergence without the need to measure abundance of individual species.

The trait-transect method is a taxon-free method to estimate CWM in herbaceous communities (Gaucherand & Lavorel 2007). It uses point quadrats along a transect to identify individual plants for trait measurement. The trait-transect method effectively captures CWM variation along environmental gradients, and yields comparable estimates to the taxon-explicit method, in particular for leaf traits (Gaucherand & Lavorel 2007). However, the method still requires considerable effort in communities with a complex vertical structure, as at each point of the transect, many individuals may be sampled.

The trait-random (TR) method is a rapid taxon-free method that has been trialled in this study. It measures the traits for the nearest rooted individual to random sampling points. The utility of this method is discussed in later sections.

**Table 1. Comparison of different methods for assessing trait values in herbaceous vegetation that can be used to quantify functional diversity**

<table>
<thead>
<tr>
<th>Method</th>
<th>Intensive methods</th>
<th>Rapid methods</th>
<th>Trade-offs: rapid cf. intensive for same effort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxon-explicit</td>
<td>Sampling populations across the range of sites for each taxon</td>
<td>Species-based (or higher taxonomic level) field measurements; using pre-existing trait data (may be population-based locally, or more generally species-based)</td>
<td>More species and/or more traits can be included in a study using rapid methods, but there can be uncertainty about intraspecific trait variation (due to genetic diversity or environmental plasticity)</td>
</tr>
<tr>
<td>Taxon-free</td>
<td>Trait-transect method</td>
<td>Trait-random method; other agronomic methods (e.g. handfuls; de Vries &amp; de Boer 1959)</td>
<td>Trait-transect method requires considerable effort in swards with complex vertical structure; rapid methods enable more area and/or more traits to be sampled for the same effort; trait-random method tends to overrepresent small suppressed plants and estimate functional divergence poorly</td>
</tr>
</tbody>
</table>

Species abundance is combined with species trait data to determine the CWM. A review of trait-based studies that have sought to quantify the functional diversity of communities indicates that the measures of species abundance used are almost entirely restricted to biomass, cover and species frequency (Supplementary Appendix S1). We discuss the main aims and methods of each and illustrate their application. We compare the most intensive methods with one or several rapid methods that can be used to reduce sampling effort and/or increase the area or number of communities sampled (Table 2).

**Biomass**

Biomass is the most relevant quantity for weighting traits correlated with immediate functions (*sensu* Grime 1998) that relate to pools and fluxes of material (e.g. biogeochemistry, flammability, palatability and nutritional quality). These have been of greatest interest to ecosystem ecologists interested in biogeochemical fluxes and agronomists interested in production. For example, specific above-ground net primary productivity (ANPP) and decomposition have been linked to CWM calculated with relative biomass (Lavorel & Garnier 2002; Garnier et al. 2004, 2007).

Harvesting, hand-sorting and weighing of vegetation is the most accurate and intensive method of sampling biomass. However, with larger plots, more replicates or additional treatments, the effort required rises rapidly to the point where labour and expense become limiting. Other drawbacks are noted by ‘t Mannetje & Jones (2000).

Rapid methods involve estimation techniques that are not as accurate as harvesting, but at the plot scale, many more quadrats can be sampled, resulting in increased precision. The dry-weight-rank method (‘t Mannetje & Haydock 1963) is a relatively simple method where taxa are ranked visually on their dry weight. Ranks for each
Table 2. Comparison of different field methods for assessing abundance of species in herbaceous vegetation that can be used to quantify functional diversity

<table>
<thead>
<tr>
<th>Measure of abundance</th>
<th>Intensive method</th>
<th>Rapid methods of sampling</th>
<th>Trade-offs: rapid cf. intensive for same effort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>Cutting, sorting and weighing all species by hand</td>
<td>Ranking of dominants by amount of biomass can be combined with estimates of total biomass against a calibrated standard</td>
<td>More area sampled or less uncertainty in estimating the contribution of individual species</td>
</tr>
<tr>
<td>Cover</td>
<td>Point quadrats, all species touched are recorded</td>
<td>Point quadrat, first touch recorded only; visual estimates; ranking of dominants</td>
<td>More area sampled but poorer estimates for contribution of minor species</td>
</tr>
<tr>
<td>Frequency</td>
<td>Presence/absence of species across multiple sampling areas</td>
<td>Reducing number or precision of taxa recorded; ‘absence frequency’</td>
<td>If fewer species are being recorded, more certainty in the estimate of frequency for those species is possible, or a greater area can be sampled. However, resilience is often highly related to functional diversity so minor species can be important</td>
</tr>
</tbody>
</table>

species are weighted by constants validated over a range of vegetation communities (e.g. Jones & Hargreaves 1979; Barnes, Odendaal & Beukes 1982). Since only the first three are typically ranked, minor species may be underestimated with this technique, although this can be offset by increasing the number of quadrats.

Botanical composition from the dry-weight-rank can be linked to biomass on a plot basis by an independent method (see ‘t Mannetje & Jones 2000 for a discussion of both destructive and non-destructive methods). However, a well-tested method is available (BOTANAL, Tothill et al. 1992) which combines the dry-weight-rank method with another visual estimate, the comparative yield method (Haydock & Shaw 1975). In each quadrat, as well as ranking species, each observer estimates herbage mass. Individual observer estimates are calibrated by regression using a small number of separate quadrats which are estimated, harvested, dried and weighed.

Relatively few of the studies reviewed (9 of the 44 found; Appendix S1, see Supplementary material) used biomass to determine CWM. This may reflect the labour intensiveness of the methods used, although two (McIntyre & Lavorel 2001; McIntyre et al. 2005) used the rapid biomass ranking technique. Biomass has been used to investigate CWM responses to succession (Garnier et al. 2004; Vile, Shipley & Garnier 2006a), grassland extensification (Garnier et al. 2007), nitrogen fertilization (Suding et al. 2005) and community resistance to exotic species invasion (Prieur-Richard et al. 2002; McIntyre et al. 2005). Only three studies used CWM traits to explain effects on immediate functions (specific ANPP, decomposition, soil C and N pools; Garnier et al. 2004, 2007; Vile, Shipley & Garnier 2006b).

Cover

Cover is the projection of plants or plant parts onto the soil surface, and can be measured as either canopy cover or basal cover (‘t Mannetje & Jones 2000). Taken as an approximation for biomass (and although this is widely recognized as contingent on canopy structure and density), cover estimates can be used for calculating functional diversity indices (Lepš et al. 2006). Strictly speaking, cover estimates are best used to address functions that depend on horizontal space occupation (e.g. resistance to invasion, evaporation, soil protection). Relevant traits associated with these include lateral spread and leaf area index.

The most precise and intensive means of measuring cover is the point quadrat (PQ) (Levy & Madden 1933; Vittoz & Guisan 2007), although other methods, such as the wheel-point method (Tidmarsh & Havenga 1955), can be used for larger areas. However, Warren-Wilson (1959) has pointed out that there are many factors to be taken into account in using point quadrats, including the precision of estimates required, the effect of pin diameter and the possible use of inclined point quadrats to overcome the differential errors in estimation of cover of species with either vertically or horizontally aligned foliage.

An important consideration in point quadrat assessments of canopy cover is whether to record only the first touch of a species (i.e. at the highest point in the canopy) or touches by all species through the canopy. Clearly, there is less effort required to only record the first touch of species; however, taller plants may be recorded preferentially. Another rapid method is to only record the dominants or to group species at the functional level (e.g. legumes). Visual estimation of cover is frequently used to decrease effort, but it is difficult to calibrate such a measure without considerable effort. Cover classes (e.g. Braun-Blanquet 1932) or ranking can be used, but as the class values are not directly related to cover, this may limit their applicability (Podani 2006).

In the reviewed studies, cover was the most common measure used to calculate CWM traits (19 of the 43 studies found). Most studies used either point quadrats or visual estimation. Cover has been used to classify vegetation patterns across environmental and disturbance gradients according to traits (Díaz et al. 1999a; Vandvik & Birks 2002) and assess the response of vegetation communities to climate change (Díaz & Cabido 1997), grassland management (Sternberg et al. 2000; Cingolani, Posse & Collantes 2005; Quétier et al. 2007) and to assess and predict restoration success (Kahmen, Poschlod & Schreiber 2002; Maurer, Durka & Stocklin 2003; Hérault, Honnay & Thoen 2005).

Frequency

The frequency of a species is estimated as the proportion of quadrats in which it is observed (‘t Mannetje & Jones 2000). Species frequency estimates are required to address ecosystem properties that depend on the number and spatial spread of individuals, generally related to resilience (e.g. disturbance, extreme events) or variability (e.g.
climate). Frequency estimates are also more suited to represent species that are not dominant, and hence poorly detected by biomass- or cover-based methods, but could have long-term ecosystem effects (Grime 1998). Stability functions, which are relevant to biodiversity dynamics and management (conservation and restoration), are hypothesized to relate to the diversity of trait values within the community (e.g. Walker, Kinzig & Langridge 1999; Mason et al. 2005). It is also hypothesized that regeneration traits are relevant to stability (Grime 2006).

When recording frequency, quadrat size must also be appropriate for the size of individuals present and density of species. Smaller quadrats are required for sampling species with higher densities, whereas much larger quadrats are required to sample rare species (Morrison, Le Brocque & Clarke 1995). There are few methods to reduce sampling time for frequency measurements apart from reductions in the completeness of taxonomic survey, such as recording only dominants, reducing the resolution of taxonomic units (e.g. genus, family) and recording only some taxonomic groups (e.g. grasses).

We found 17 published studies using frequency, typically obtained from species presence or absence in quadrats or sites. Frequency provides a widely applicable and unitless measure of abundance that is particularly useful for comparing traits across different floras (i.e. meta-analyses) where the original data sets may not have been collected for that purpose. Frequency has been used to assess trait responses of communities to disturbances such as grazing (e.g. Díaz, Noy-Meir & Cabido 2001; de Bello et al. 2005; del Pozo et al. 2006) or climate (e.g. Díaz et al. 1999b), succession (Dahlgren et al. 2006) and land use change (Verheyen et al. 2003).

Case study

STUDY SITE

The Lautaret study site was located on the south-facing aspect of the upper valley of the Romanche River, central French Alps (Villar d’Arène, 45·04°N, 6·34°E). (see Quétier et al. 2007 for site details). We used 12 plots representing five land use treatments (Quétier et al. 2007). These included: (i) three fertilized hay meadows on previously cultivated terraces; (ii) three unfertilized hay meadows on previously cultivated terraces; (iii) one unfertilized and unmown but lightly grazed terrace; (iv) three never ploughed hay meadows; and (v) two former hay meadows on previously cultivated terraces; (iii) one unfertilized and unmown but lightly grazed terrace; (iv) three never ploughed hay meadows; and (v) two former hay meadows that are no longer mown but lightly grazed in summer. These communities are perennial, resprout each year after snowmelt from buried buds and are dominated by graminoids (45%–80% of total cover). Because recruitment of new species is rare (Zeiter, Stampfli & Newbery 2006), we assumed that interannual compositional turnover is negligible.

We measured five traits previously shown to capture functional variations across land use treatments (Quétier et al. 2007): vegetative and reproductive height, leaf dry matter content (LDMC) and leaf nitrogen content (LNC), and seed mass. Four main methods were applied to quantify CWM and functional divergence (Table 3).

<table>
<thead>
<tr>
<th>Trait measurement method</th>
<th>Abundance method</th>
<th>Taxon-explicit</th>
<th>Taxon-free</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>Intensive</td>
<td>Point quadrat (PQ; 2003)</td>
<td>Trait-transect (2006)*</td>
</tr>
<tr>
<td></td>
<td>Rapid</td>
<td>Trait-random (TR; 2006)</td>
<td>Not measured</td>
</tr>
<tr>
<td>Biomass</td>
<td>Intensive</td>
<td>Hand sorting (HS; 2004)</td>
<td>Not measured</td>
</tr>
<tr>
<td></td>
<td>Rapid</td>
<td>Dry-weight Rank – BOTANAL (DWR; 2006)</td>
<td>Not measured</td>
</tr>
</tbody>
</table>

*See previous section for descriptions of types of trait and abundance measurements. Abbreviations for each method are used in the text and illustrations.
†The trait-transect method was only applied for three plots for comparison of sampling effort and precision with the TR method.

biomass cuts within each plot were hand-sorted to species. Methods for these two sets of measurements can be found in Quétier et al. (2007).

Third, in July 2006, a rapid assessment of total biomass per metre square and species relative contributions to total green biomass was made using BOTANAL (Jones & Hargreaves 1979). We sampled eighty 50 × 50 cm quadrats per plot. Visual estimates of total and fraction green biomass were made by a single observer and calibrated against 20 harvested quadrats. These were sorted into green and litter parts, oven dried at 60 °C until constant mass and weighed. The calibration between estimated and measured biomass was satisfactory ($R^2 = 0.748$, $P < 0.01$). In each quadrat, the three most abundant species were ranked by biomass, using consensus estimates between two observers. Standard weightings to ranks were used to estimate biomass of each species per plot (Jones & Hargreaves 1979).

For each of these three methods, CWM and functional divergence for each trait were derived by combining species relative abundances with population-based trait measures (Shimatani & Lavorel (2007)).

CWM was calculated as:

$$\text{CWM} = \sum_{i=1}^{s} p_i \times \text{trait},$$

where $p_i$ is the relative contribution of species $i$ to the community, and trait is the trait value of species $i$.

Functional divergence was estimated for each trait using the Rao coefficient modified by Leps et al. (2006), which captures intraspecific (or intrapopulation when using population-level trait values) as well as interspecific (or interpopulation when using population-level trait values) variability in traits. If the proportion of $i$th species in a plot is $p_i$ and dissimilarity of species $i$ and $j$ is $d_{ij}$, the Rao coefficient estimates functional divergence as:

$$\text{FD} = \sum_{i=1}^{s} \sum_{j=1}^{s} d_{ij} p_i p_j$$

where $s$ is the number of species in the community (Shimatani 2001). Dissimilarity among species $d_{ij}$ was calculated as $d_{ij} = 1 - O_{ij}$, where $O_{ij}$ is the overlap between the probability density functions for the trait values of species $i$ and $j$. $O_{ij}$ was estimated using the normal approximation for each of the functions. The actual value was calculated numerically using an Excel Macro (http://botanika.bf.jcu.cz/suspa/FunctDiv.php).

STATISTICAL ANALYSES

The comparison was conducted in three steps:

1. Robustness of population trait values across years was tested by regression and Pearson correlation tests.
2. We compared plot-level estimates yielded by the four methods for CWM and functional divergence using regression and Pearson correlation tests.
3. We compared land use treatment-level estimates yielded by the four methods for CWM and functional divergence. To accommodate for uneven numbers of replicate plots per treatment, we used Residual Maximum Likelihood methods (REML) to test the combined effects on CWM and functional divergence of treatments and methods, taken as categorical factors with random effects. This analysis sought in particular to test for interaction terms, which represent differences in treatment rankings across methods. All analyses were conducted using GenStat® 8:1 (GenStat 2005).

Congruency among methods was used as a criterion for the evaluation of each of the two rapid methods (DWR Botanal and TR) against the more intensive methods (Hand Sorting [HS] and PQ).

RESULTS

ROBUSTNESS ACROSS YEARS OF POPULATION TRAIT VALUES

Vegetative height was well correlated between 2006 and 2003 ($R^2 = 0.789$, $P < 0.001$), although plants were on average 5 cm shorter than in 2003 (Supplementary Fig. S1a). Reproductive height was conserved across the two years ($R^2 = 0.847$, $P < 0.001$), with no significant difference in absolute values as shown by the identity of the regression with a 1 : 1 line (Supplementary Fig. S1b). LDMC was also stable across years ($R^2 = 0.840$, $P < 0.001$), though slightly lower in 2006 than in 2003 (Supplementary Fig. S1c). Accordingly, Leaf Nitrogen Content, although stable across years ($R^2 = 0.519$), was slightly higher in 2006 than in 2003 (Figure S1d).
A previous comparison for the Lautaret grasslands also demonstrated that species-level trait estimates taken across 16 common species between 2004 and 2003 (eight grasses, six dicots and two legumes) were moderately well correlated for vegetative and reproductive heights ($R^2 = 0.627$, $P = 0.03$; and $R^2 = 0.558$, $P = 0.05$), respectively, with shorter plants in 2004 than in 2003. Likewise, species-level LDMC was correlated across the 2 years ($R^2 = 0.550$, $P = 0.05$) and lower in 2004, and LNC was conserved ($R^2 = 0.718$, $P < 0.005$) (A. Thébault, unpubl. data). These and our findings confirm previous assessments that demonstrated the robustness of leaf traits and functional classifications to interannual variability (Garnier et al. 2001; Roche et al. 2004; Foster & Brooks 2005).

Based on these results, we were confident that trait values measured in 2003 could be used to estimate functional diversity in 2006. Regression equations across years were hence used to calibrate new measurements and combine them with the 2003 data base.

**EFFECTS OF METHODOLOGY ON ESTIMATES OF COMMUNITY-WEIGHTED MEAN TRAITS**

Total and green biomass estimates obtained using the BOTANAL method in 2006 were within the range of values measured by hand-sorting in 2004 and 2005 on the same plots (data not shown). Analysis of year and treatment effects showed that, in spite of interannual climatic variation, there was little among-year variation in total (REML Wald statistics $R^2 = 0.61$), nor was there a significant interaction between land use treatments and years (total biomass, Wald statistic $= 1.69$, $P = 0.094$; green biomass Wald statistic $= 0.56$, $P = 0.69$).

The three methods for estimation of relative abundances (Dry Weight Ranks [BOTANAL], PQ and HS) produced similar ranks for the five most dominant species within each treatment, representing from 70% (treatment 1, species-rich mown and fertilized) to 90% or more (treatments 3 and 5, lightly grazed with strong dominance by a few grass species) of cumulated abundance. BOTANAL tended to systematically overestimate relative abundances of the two to three most dominant species by up to 10%, with the greatest effect in the most diverse communities. As expected, biomass had a steeper biomass rank curve than cover (data not shown). Estimates of CWM for vegetative height, reproductive height, LDMC and LNC were consistent across the three methods estimating relative abundances (HS, PQ and BOTANAL) (significant pairwise correlations, Table 4; Fig. 1). BOTANAL estimates for CWM LDMC and LNC were well correlated with estimates from biomass sorting data (Table 4, Fig. 1a,b). BOTANAL-based estimates of vegetative height showed no systematic bias (no departure from the 1 : 1 line), whereas reproductive height showed a systematic bias of +5 to 10 cm (Fig. 1c,d). CWM seed mass estimates on the other hand showed poor correlation across methods (Table 4, Fig. 1e).

The TR method provided fair estimates in relation to the other three methods for LDMC (Table 4). Estimated values were well correlated with BOTANAL estimates, although there was a tendency towards lower estimates by TR on higher LDMC plots as shown by an improved fit with a quadratic regression ($y = -0.026x^2 + 17.8x - 2636$, $R^2 = 0.810$). The TR method apparently overrepresented dominated

**Table 4.** Pairwise correlations across field methods used to calculate CWM for five traits at Lautaret (abbreviations for methods are from Table 3)

<table>
<thead>
<tr>
<th>Methods (x–y)</th>
<th>$R^2$</th>
<th>$P$ value</th>
<th>S ± SE</th>
<th>I ± SE</th>
<th>$R^2$</th>
<th>$P$ value</th>
<th>S ± SE</th>
<th>I ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Vegetative height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS–PQ</td>
<td>0.966</td>
<td>&lt; 0.001</td>
<td>0.94 ± 0.07</td>
<td>3.0 ± 1.8</td>
<td>0.880</td>
<td>&lt; 0.001</td>
<td>0.81 ± 0.11</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>PQ–BOTANAL</td>
<td>0.894</td>
<td>&lt; 0.001</td>
<td>1.02 ± 0.13</td>
<td>1.6 ± 3.7</td>
<td>0.774</td>
<td>&lt; 0.01</td>
<td>1.05 ± 0.21</td>
<td>-4 ± 13</td>
</tr>
<tr>
<td>PQ–TR</td>
<td>0.476</td>
<td>0.039</td>
<td>1.44 ± 0.57</td>
<td>9.6 ± 7.2</td>
<td>0.215</td>
<td>NS</td>
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<td></td>
</tr>
<tr>
<td>HS–BOTANAL</td>
<td>0.863</td>
<td>&lt; 0.001</td>
<td>0.95 ± 0.14</td>
<td>14 ± 3.9</td>
<td>0.774</td>
<td>&lt; 0.01</td>
<td>0.91 ± 0.18</td>
<td>13 ± 10</td>
</tr>
<tr>
<td>HS–TR</td>
<td>0.452</td>
<td>0.047</td>
<td>0.31 ± 0.13</td>
<td>4.1 ± 3.5</td>
<td>0.056</td>
<td>NS</td>
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</tr>
<tr>
<td>BOTANAL–TR</td>
<td>0.72</td>
<td>&lt; 0.01</td>
<td>0.38 ± 0.09</td>
<td>2.2 ± 2.4</td>
<td>0.035</td>
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<tr>
<td>(c) Leaf dry matter content</td>
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<tr>
<td>HS–PQ</td>
<td>0.842</td>
<td>&lt; 0.001</td>
<td>0.82 ± 0.13</td>
<td>67 ± 46</td>
<td>0.899</td>
<td>&lt; 0.001</td>
<td>0.81 ± 0.10</td>
<td>2.7 ± 1.7</td>
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<tr>
<td>PQ–BOTANAL</td>
<td>0.741</td>
<td>&lt; 0.01</td>
<td>1.34 ± 0.30</td>
<td>107 ± 104</td>
<td>0.786</td>
<td>&lt; 0.001</td>
<td>1.09 ± 0.20</td>
<td>-2.5 ± 3.5</td>
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<tr>
<td>PQ–TR</td>
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<td>0.73 ± 0.16</td>
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<td>0.075</td>
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<td>1.4 ± 4.1</td>
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<td>0.100</td>
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<td>0.41 ± 0.13</td>
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<td>0.021</td>
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<td>PQ–BOTANAL</td>
<td>0.346</td>
<td>0.074</td>
<td>-0.94 ± 0.46</td>
<td>0.84 ± 0.21</td>
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<tr>
<td>PQ–TR</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>BOTANAL–TR</td>
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<td></td>
<td></td>
<td></td>
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</tbody>
</table>

NS, not significant; S, slope; I, intercept; SE, standard error.

(shaded) individuals, especially in the tall, unmown grasslands with high LDMC (treatment 5). Estimates of CWM vegetative height were consistent with other methods, and moderately well correlated with BOTANAL-based estimates, though much lower (from 10 cm in grazed meadows with a lower sward to 20 cm in the tallest grasslands; Supplementary Fig. S2). Estimates of reproductive height were poor in comparison to all other methods (Table 4), probably due to the low number of flowering individuals sampled. Overall, concentrating on nearest rooted individuals rather than all individuals touching the pin enhanced the bias detected for the trait-transect method towards individuals smaller than the ‘well-grown’ standard (Gaucherand & Lavorel 2007). A complementary test in three plots with increasing sward height and complexity (in treatments 3, 1 and 5) confirmed that the trait-transect method applied for an equal number of sampling points estimated higher CWM vegetative heights, and especially so for taller swards, but that they remained below BOTANAL estimates for the same plots (by 20 cm) (data not shown).

REML analyses of the combined effects of land use treatments and method confirmed the stability of CWM with respect to method (Table 5, Supplementary Fig. S2). CWM LNC was the least sensitive to method, with a significant response to land use but no effects of method, but was not estimated for the TR method. Due to a consistent difference between the TR and other methods, estimates of CWM LDMC and reproductive height were sensitive to method, but there was no interaction with treatment effects (Supplementary Fig. S2). So all methods could be used interchangeably to detect land use effects on CWM for leaf traits. Vegetative height showed a similar behaviour, although response to land

Fig. 1. Regression between estimates of CWM using the BOTANAL method (y-axis) vs. the hand sorting method (x-axis) for: (a) vegetative height, (b) reproductive height, (c) leaf dry matter content (LDMC), (d) leaf nitrogen content (LNC) and (e) seed mass. Each point represents one plot for which CWM were estimated with the two methods. The regression line and its equation, and $R^2$ are inserted in each graph. The dashed line represents a 1 : 1 relationship to which the significant regression is compared.
use was less sensitive with TR than with other methods (significant land use × method interaction). Finally, differences in CWM seed mass across land use treatments were detected across the three taxon-explicit methods. They were strong and consistent for the HS method and BOTANAL, but PQ estimates were more variable across plots.

In conclusion, we detected little sensitivity of CWM estimates to method. Congruency across methods was greatest for leaf traits, followed by heights, and was only moderate for seed mass. Of the two rapid methods tested, BOTANAL appeared as a more reliable method to estimate CWM for traits of the vegetative phase (heights and leaf traits) because it was most congruent with other more time-consuming methods (PQ and HS).

### Effects of Methodology on Estimates of Functional Divergence

The three methods for relative abundance estimates (HS, PQ and BOTANAL) produced fairly consistent estimates of functional divergence for leaf and height traits (Table 6), although correlations among methods were less strong than for CWM. Estimates by BOTANAL were overall less well correlated with HS or PQ estimates than for CWM (Table 6), but responses to land use treatments were consistent between the three methods (Table 5, Supplementary Fig. S3). Estimates of functional divergence through the TR method on the other hand were not congruent with the other three methods (Table 6). They often produced lower values (Supplementary Fig. S3), and were unable to detect land use treatment differences (significant treatment × method interaction, Table 5). Functional divergence estimates through the TR method were particularly poor for reproductive height as several quadrats had no or too few flowering individuals sampled.

Estimates of functional divergence for seed mass were consistent between the HS and PQ methods, but not with BOTANAL, which tended to produce lower values, especially than the cover-based method (Table 6, Supplementary Fig. S3). However, the three methods consistently detected differences across treatments (Table 5; no significant treatment × method interaction). We confirm that overall functional divergence is much more sensitive than CWM to the method used to estimate relative abundances (Lepš et al. 2006) and/or traits (TR method).

### So, does methodology matter?

While there has been much work developing standard protocols for estimating plant traits, most field studies do not explicitly address the effects of trait and species abundance measurements on estimates of functional diversity. We have shown that a more systematic exploration of how methods influence the accuracy of functional diversity estimates is needed, and this should be considered when designing field studies. We now return to the three questions raised in the introduction of this paper.

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**Table 5.** REML analysis of the combined effects of assessment method and land use treatment on estimates of CWM (left section) and functional divergence (right section) at Lautaret: (a) vegetative height, (b) reproductive height, (c) leaf dry matter content (LDMC), (d) leaf nitrogen content (LNC) and (e) seed mass

<table>
<thead>
<tr>
<th>Method/df</th>
<th>Wald statistic</th>
<th>df</th>
<th>P</th>
<th>Method/df</th>
<th>Wald statistic</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Vegetative height</td>
<td>Method</td>
<td>299.07</td>
<td>3</td>
<td>&lt;0.001</td>
<td>Functional divergence</td>
<td>22.55</td>
<td>3</td>
</tr>
<tr>
<td>Treatment</td>
<td>419.73</td>
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<td>&lt;0.001</td>
<td>Treatment</td>
<td>71.35</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Method × treatment</td>
<td>62.64</td>
<td>12</td>
<td>&lt;0.001</td>
<td>Method × treatment</td>
<td>20.34</td>
<td>12</td>
<td>0.061</td>
</tr>
<tr>
<td>(b) Reproductive height</td>
<td>Method</td>
<td>83.77</td>
<td>3</td>
<td>&lt;0.001</td>
<td>Method</td>
<td>24.16</td>
<td>3</td>
</tr>
<tr>
<td>Treatment</td>
<td>56.95</td>
<td>4</td>
<td>&lt;0.001</td>
<td>Treatment</td>
<td>18.85</td>
<td>4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Method × treatment</td>
<td>11.87</td>
<td>12</td>
<td>0.456</td>
<td>Method × treatment</td>
<td>46.43</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(c) LDMC</td>
<td>Method</td>
<td>27.69</td>
<td>3</td>
<td>&lt;0.001</td>
<td>Method</td>
<td>24.38</td>
<td>3</td>
</tr>
<tr>
<td>Treatment</td>
<td>69.09</td>
<td>4</td>
<td>&lt;0.001</td>
<td>Treatment</td>
<td>30.01</td>
<td>4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Method × treatment</td>
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<td>12</td>
<td>0.609</td>
<td>Method × treatment</td>
<td>10.45</td>
<td>12</td>
<td>0.577</td>
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<tr>
<td>(d) LNC</td>
<td>Method</td>
<td>3.18</td>
<td>2</td>
<td>0.204</td>
<td>Method</td>
<td>3.92</td>
<td>2</td>
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<tr>
<td>Treatment</td>
<td>113.63</td>
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<td>&lt;0.001</td>
<td>Treatment</td>
<td>12.2</td>
<td>4</td>
<td>0.016</td>
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<tr>
<td>Method × treatment</td>
<td>3.98</td>
<td>8</td>
<td>0.859</td>
<td>Method × treatment</td>
<td>5.38</td>
<td>8</td>
<td>0.716</td>
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<tr>
<td>(e) Seed mass</td>
<td>Method</td>
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<td>0.372</td>
<td>Method</td>
<td>23.95</td>
<td>2</td>
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<tr>
<td>Treatment</td>
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</tr>
<tr>
<td>Method × treatment</td>
<td>5.92</td>
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<td>0.656</td>
<td>Method × treatment</td>
<td>8.14</td>
<td>8</td>
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SHOULD THE OBJECTIVE OF THE STUDY INFLUENCE THE METHOD FOR ESTIMATING RELATIVE ABUNDANCE?

We considered three metrics to assess plant abundance in herbaceous vegetation: biomass, cover and frequency. We suggest that they differ in their suitability in detecting the range of different functions that might be of interest to a particular study. We recognize that different functions relate to different groups of species within a community (Grime 1998, 2006), and that this needs to direct the choice of metrics. Our review of published studies that have measured functional diversity in the field showed that metrics are usually chosen without consideration of their links with the functions of interest. Most studies have focused on identifying responses of CWM to environmental change (e.g. disturbance, grazing and climate change) and have chosen cover to estimate abundance. This would appear appropriate granted that aspects of horizontal space occupation can be related to response to environmental perturbations. Frequency may also be a relevant method, especially for communities with high evenness. The paucity of studies utilizing biomass may reflect both the time-consuming nature of such methods and research questions. Relating functional diversity estimates to immediate functions is a growing research interest (Petchey & Gaston 2006). For that purpose, shifting practice from frequency and cover measurements to biomass estimates may be warranted. Rapid methods, including the methods used in this study, are well established and have long history in agronomic research, but functional ecologists are generally unaware of them. They could be a viable, cost-effective alternative to biomass sorting. Encouragingly, our case study also suggests that cover can provide a robust estimate of CWM for vegetative traits. On the other hand, frequency may bias CWM estimates as it tends to overestimate the contribution of subordinate species. The situation is unfortunately more complex for estimating CWM for regeneration traits, and for estimating functional divergence for all traits. We showed that CWM seed mass and functional divergence can be sensitive to abundance metrics. Ultimately, CWM and functional divergence depend on the distribution of trait values among the more abundant species within the community (because they use abundance weightings). Vegetative traits such as height and especially leaf traits tend to be filtered within communities (i.e. values are similar among dominants; Grime 2006). Therefore, small variations across metrics in their abundance estimates do not influence the calculated CWM, or functional divergence in the case of leaf traits. Regeneration traits on the other hand are often variable among dominants (Grubb 1986; Grime 2006), and this leads to instability of functional diversity measures depending on the method used to weight their traits in the calculation of indices. This instability is therefore constitutional and should be borne in mind when applying and interpreting functional diversity measures for regeneration traits.

WHAT ARE THE STRENGTHS AND LIMITATIONS OF INTENSIVE VS. RAPID APPROACHES, AND WHEN SHOULD EITHER BE APPLIED?

Taxon-explicit trait measurements

Trait data bases and floras are widely used to provide trait data for calculations of functional diversity. Their use reduces the time and effort required to undertake such studies, and/or

<table>
<thead>
<tr>
<th>Methods</th>
<th>$R^2$</th>
<th>$P$</th>
<th>$S \pm SE$</th>
<th>$I \pm SE$</th>
<th>$R^2$</th>
<th>$P$</th>
<th>$S \pm SE$</th>
<th>$I \pm SE$</th>
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</thead>
<tbody>
<tr>
<td>(a) Vegetative height</td>
<td></td>
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<td></td>
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<td>(b) Reproductive height</td>
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<tr>
<td>HS–PQ</td>
<td>0.908</td>
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<td>1.01 ± 0.12</td>
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<td>0.417</td>
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<td>0.74 ± 0.33</td>
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<td>0.655</td>
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<td>0.50 ± 0.18</td>
<td>0.25 ± 0.1</td>
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<td></td>
<td></td>
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<tr>
<td>HS–BOTANAL</td>
<td>0.542</td>
<td>0.024</td>
<td>0.90 ± 0.31</td>
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<td>0.494</td>
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<td></td>
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<td>0.302</td>
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<td></td>
<td></td>
<td>0.549</td>
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<td>−1.10 ± 0.50</td>
<td>1.05 ± 0.26</td>
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<tr>
<td>(c) LDMC</td>
<td></td>
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<td></td>
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<td>(d) LNC</td>
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<tr>
<td>HS–PQ</td>
<td>0.554</td>
<td>0.021</td>
<td>0.63 ± 0.21</td>
<td>0.18 ± 0.10</td>
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<td>1.21 ± 0.33</td>
<td>−0.06 ± 0.17</td>
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<tr>
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<td>(e) Seed mass</td>
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<td>HS–PQ</td>
<td>0.627</td>
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<td>0.52 ± 0.14</td>
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<tr>
<td>HS–TR</td>
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<tr>
<td>BOTANAL–TR</td>
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<td></td>
<td></td>
<td></td>
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</tbody>
</table>

NS, not significant; S, slope; I, intercept; SE, standard error.
increases the number of species or traits that can be considered. Spatial and temporal variations in trait values within species may limit the use of data bases, but this has not been tested beyond assessments of the conservation of species rankings (Garnier et al. 2001; Roche et al. 2004). Our case study suggested that there was consistency of trait measurements at population and to some extent species level among years. Good practice for use of pre-existing trait data should involve some testing and calibration as described in our robustness analysis above. Furthermore, although current standards suggest that trait data should be collected using population-centred protocols, trait data bases apply at a species level when used at sites other than those where the original data was collected. A comparison of CWM vegetative height, reproductive height, LDMC and LNC calculated using either population-level or average site-level (mean across populations) trait values for Lautaret showed that they were strongly similar ($R^2 = 0.881$ for vegetative height, $0.947$ for reproductive height, $0.941$ for LDMC, $0.955$ for LNC; no departure from the $1:1$ line for any of these traits). This would need to be confirmed for more sites and may not apply everywhere (Diaz et al. 1999b).

Temporal and spatial variation in trait values will introduce two sources of uncertainty for estimates of functional diversity when they affect trait values among dominants. We showed that these effects may be strongest for functional divergence. Effects of intraspecific trait variability will depend on the magnitude of differences of interspecific differences. In many cases, intraspecific variability has been shown to be smaller than interspecific variability (Garnier et al. 2001), but intraspecific studies also remind us that traits can vary considerably within species, due to both plasticity (e.g. Puijalon & Bornette 2004; Al Haj Khaled et al. 2005; Semmarten & Ghersa 2006) and genetic differences between populations (Marron et al. 2005).

Rapid assessments of species abundances

Estimating species abundance can be time intensive, preventing assessments at broad spatial scales or repeated measurements through time. Each method represents a point on a three-way continuous trade-off between number of species, area and time. Rapid methods have substantial advantages as the area sampled increases. Although sampling error for individual measurement units may be greater, the among-sample/treatment error should decline with increasing numbers of samples. By covering a larger area, rapid methods can sample more species, especially less frequent ones.

To assess functional diversity through CWM and functional divergence, precision in assessing the more abundant species matters most. Rapid methods will therefore be of greatest benefit if they conserve species abundance rankings and do not introduce systematic bias. However, rapid methods may perform poorly in vegetation communities with even abundances, whether using biomass or cover.

Our field trial (using BOTANAL) suggested that rapid visual estimates of biomass and dominance ranks assessed functional diversity well when compared to the far more time-consuming HS method. Given that BOTANAL took a fifth of the time, can we confidently switch current practice? BOTANAL satisfactorily detected responses to the grassland management treatments but can skew abundance estimates towards the most dominant species if sampling intensity (i.e. number of quadrats) is too low. However, systematic comparisons of HS with BOTANAL have demonstrated that it provides a reliable representation of abundances for all but infrequent species, which are never dominants (Jones & Hargreaves 1979). Loss of information on less-frequent species does not affect estimates of CWM and functional divergence unless they have a common trait value that differs from the dominants (e.g. rare and subordinate species in alpine meadows with high per capita N uptake; Theodose et al. 1996). In grasslands, the less-abundant species may be important to community resilience or response to climatic variation. In contrast to the dominant grasses, an array of forbs and smaller grasses provide high diversity (Grubb 1986; Trémont 1994) and present a range of different traits that contribute to varying environmental response (McIntyre & Lavorel 2001). In our case study, BOTANAL was particularly reliable in estimating CWM. BOTANAL was also suitable to measure functional divergence. However, care should be taken in the use of any method because functional divergence appeared sensitive to all the methods used for abundance estimates.

ARE TAXON-FREE METHODS ROBUST IN COMPARISON TO TAXON-EXPLICIT METHODS OF TRAIT MEASUREMENT?

Although we have contrasted taxon-explicit and taxon-free approaches, there is in practice a continuum between them. One frequently used approach to reduce sampling time is to simplify trait estimates by collecting data at a higher taxonomic level than species or populations. This will have little effect on estimates of functional diversity for traits with low interspecific variation within higher taxonomic groups – often morphological or leaf structural traits. Effects on other traits will only matter if their values are significantly different among species, and these species have high abundances. This may be particularly problematic for higher-level groupings, including aggregation to the level of life-forms (Wright et al. 2006).

The taxon-free TR method examined in our field study reliably estimated leaf traits (LDMC and LNC) but consistently biased estimates of CWM towards low values and was less sensitive to treatments than the three taxon-explicit methods. Functional divergence estimates were consistent with other methods in capturing treatment responses of leaf traits, but absolute values were underestimated. These patterns are probably due to the sampling method, which overrepresented species with large basal area (e.g. stoloniferous grasses with large numbers of small stems). This bias was strongest for structurally complex communities and confirmed trends observed using the trait-transect method (Gaucherand & Lavorel 2007). The reliability of taxon-free methods that
measure traits from randomly selected individuals may therefore be strongly influenced by the size, structure and architecture of the sward and may be most applicable to communities with simple structures (e.g. even swards of stoloniferous grasses).

Botanical skills may not be required for all applications. Some common agronomic indicators of pasture condition are taxon-free assessments of CWM (e.g. sward height and herbage dry matter content). However, their use may be limited to monitoring rather than contributing to our fundamental understanding of vegetation diversity and function. The lack of vegetation composition data may indeed hinder the interpretation of results and the assessment of functional diversity for additional traits not accessible at the time of observation (i.e. seed mass), or sourced from external data bases.

Conclusions

Based on a review of main methods used for estimating functional diversity in the field and our case study, we conclude the following in relation to the choice of sampling methods in this expanding research area:

1. Previously measured (data base) values of traits can be used to estimate functional diversity, but these data need to be verified against variability between populations and/or between years due to site and seasonal variation.

2. Theoretical questions should form the basis for choices of appropriate methods for estimating relative abundance. We did not exhaustively assess all methods in the field but suggest that good practice should involve tailoring the method to the traits assessed and questions posed. Biomass is useful for some questions, but has a major cost in trading precision against area sampled.

3. We detected little sensitivity of CWM to method of estimating abundance. Congruency across methods was greatest for leaf traits, followed by height, but was only moderate for seed mass. Estimates of functional divergence, however, are more sensitive to the method used to estimate relative abundances and/or traits.

4. Methodology does matter, and care is required in the application of rapid methods for estimating abundance and trait values. When focusing on small spatial scales and detailed processes, the more intensive methods may still be best. However, rapid methods, used by agronomists for several decades, in particular BOTANAL, provide considerable promise for sampling large areas and conducting studies with repeated measures.

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Supplementary material

The following supplementary materials are available for this article:

**Appendix S1.** Results of a literature search for studies inves-

tigating aggregated plant traits and community responses.

**Fig. S1.** Regression between measurements of trait values in
2003 and 2006 trait for 23 populations. Each point represents
one population measured in the 2 years. The regression line
and its equation and $R^2$ are inserted in each graph. The dashed
line represents a 1 : 1 relationship to which the significant
regression is compared.

**Fig. S2.** Treatment means and standard error for community-
weighted mean traits estimated using the four assessment
methods (each bar represents one method) for: (a) vegetative
height, (b) reproductive height, (c) leaf dry matter content
(LDMC), (d) leaf nitrogen content (LNC) and (e) seed
mass.

**Fig. S3.** Treatment means and standard error for functional
divergence estimated using the four assessment methods (each
bar represents one method) for: (a) vegetative height, (b) repro-
ductive height, (c) leaf dry matter content (LDMC), (d) leaf
nitrogen content (LNC) and (e) seed mass.

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