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SHORT COMMUNICATION

Two Measurement Methods of Leaf Dry Matter Content Produce Similar Results in a Broad Range of Species

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• *Background and Aims* Leaf dry matter content (LDMC) is widely used as an indicator of plant resource use in plant functional trait databases. Two main methods have been proposed to measure LDMC, which basically differ in the rehydration procedure to which leaves are subjected after harvesting. These are the 'complete rehydration' protocol of Garnier *et al.* (2001, *Functional Ecology* **15**: 688–695) and the 'partial rehydration' protocol of Vendramini *et al.* (2002, *New Phytologist* **154**: 147–157).
• *Methods* To test differences in LDMC due to the use of different methods, LDMC was measured on 51 native and cultivated species representing a wide range of plant families and growth forms from central-western Argentina, following the complete rehydration and partial rehydration protocols.
• *Key Results and Conclusions* The LDMC values obtained by both methods were strongly and positively correlated, clearly showing that LDMC is highly conserved between the two procedures. These trends were not altered by the exclusion of plants with non-laminar leaves. Although the complete rehydration method is the safest to measure LDMC, the partial rehydration procedure produces similar results and is faster. It therefore appears as an acceptable option for those situations in which the complete rehydration method cannot be applied. Two notes of caution are given for cases in which different datasets are compared or combined: (1) the discrepancy between the two rehydration protocols is greatest in the case of high-LDMC (succulent or tender) leaves; (2) the results suggest that, when comparing many studies across unrelated datasets, differences in the measurement protocol may be less important than differences among seasons, years and the quality of local habitats.

Key words: Argentina, leaf dry matter content, leaf rehydration, plant comparative ecology, plant functional traits.

INTRODUCTION

Leaf dry matter content (LDMC, the ratio of leaf dry mass to fresh mass) is increasingly used as an indicator of a plant species' resource use strategy, i.e. its position in a fundamental trade-off between a rapid assimilation and growth at one extreme, and efficient conservation of resources within well-protected tissues at the other (Wilson *et al.*, 1999; Garnier *et al.*, 2001; Díaz *et al.*, 2004).

Garnier *et al.* (2001) and Vile *et al.* (2005) highlighted the importance of standardizing protocols for the measurement of LDMC. Particular emphasis was put on achieving full rehydration of leaves after their collection in the field. Although the complete rehydration method is highly desirable, it is not always practical, and other methods are still widely used in comparative ecology (see Table 1 in Vile *et al.*, 2005). The aim of this study was to compare the results of using two different methods of assessing LDMC: on the one hand, the complete rehydration method proposed by Garnier *et al.* (2001), and on the other hand a partial rehydration method utilized, with some variations, in many studies (e.g. Wilson *et al.*, 1999; Vendramini *et al.*, 2002; Prior *et al.*, 2003). In order to achieve this, LDMC was measured on 51 species belonging to 17 different families, and representing a wide range of growth forms, using both methods.

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MATERIALS AND METHODS

A set of 51 native and introduced species from central-western Argentina was considered (listed in the Appendix), representing a wide range of taxonomic families, growth forms and leaf types. Thirty-seven were in common with the dataset of Vendramini *et al.* (2002). Fourteen native and cultivated species were added to this original data set, since in the case of Vendramini *et al.*'s (2002) study, the main aim was to characterize abundant species of this region; while here, the aim was to test differences in LDMC due to the use of different measurement methods, across the widest possible range of leaf types (e.g. differences in leaf structure, toughness, size, venation, habitats of origin, etc.). All material was collected from the field during the growing season (December–March). At least four fully expanded young leaves, free from herbivore or pathogen damage, were collected from at least six randomly selected sexually mature individuals. In the case of leaf succulents or in general plants whose photosynthetic organs were not laminar leaves, the specific procedures described by Vendramini *et al.* (2002) were followed. LDMC was determined following the protocols of Garnier *et al.* (2001) and Vendramini *et al.* (2002). The basic difference is in the rehydration procedure. In the protocol of Garnier *et al.* (2001), samples are immediately placed into tubes with the cut end submerged in deionized water and stored in the dark at 4 °C for 24 h. In the case of the method of

Vendramini *et al.* (2002), samples are stored in sealed plastic bags (which are moistened in the case of mesophytic species, but not succulent and resinous species), kept at 4 °C in the dark, and measured as soon as possible (usually within 7–12 h). This method is a modification of that of Wilson *et al.* (1999), who also kept cut leaves in sealed plastic bags but promoted rehydration by storing leaves overnight between sheets of damp paper towel. Then, in both the complete rehydration and partial rehydration procedures, samples were blotted dry to remove any surface water, weighed and oven-dried in paper bags at 60 °C for at least 2 d, after which their dry mass was measured. On the basis of Garnier *et al.*'s (2001) work, full rehydration of the leaves was assumed, rather than specifically tested.

Measurements of LDMC carried out with the two methods were compared by using Pearson correlation analyses and standardized major axis (SMA) slope-fitting on $\log_{10}$ transformed data, which were back-transformed in Fig. 1, for easier visualization. SMA slope-fitting techniques are appropriate for testing if two methods of measurement agree, and in particular for testing whether measurements carried out with one method scale isometrically with measurements carried out with another method, in which case data obtained with the two methods can be mixed (Warton *et al.*, 2006). This is achieved by testing whether the slope of the fitted line is significantly different from 1 and its intercept is significantly different from 0. The SMATR 2·0 freeware (Falster *et al.*, 2006) was used for the SMA analyses.

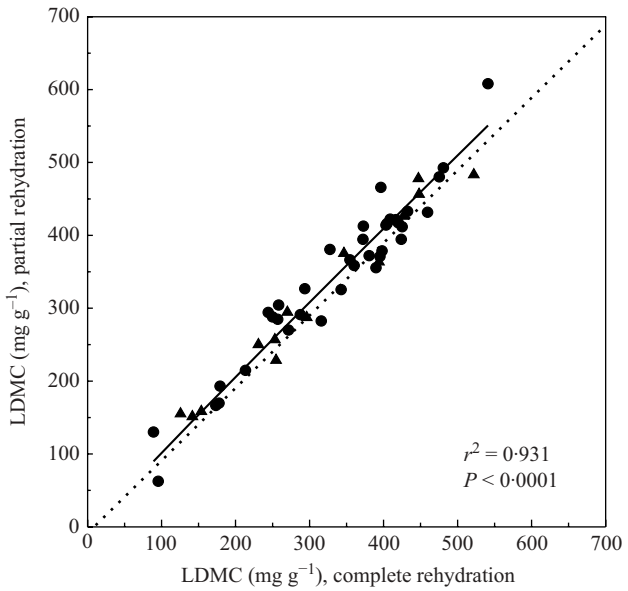

FIG. 1. Relationship between LDMC values obtained for a wide range of species using the methods of Garnier *et al.* (2001; complete rehydration) and Vendramini *et al.* (2002; partial rehydration) on 51 native and cultivated species of central-western Argentina, including the 37 used by Vendramini *et al.* (2002), indicated by closed circles. The trend line corresponds to the back-transformed SMA equation for full dataset: $\log_{10}(\text{LDMC}_{\text{partial rehydration}}) = 0{\cdot}1021 + 0{\cdot}9675[\log_{10}(\text{LDM}_{\text{complete rehydration}})]$; CI 95 % for slope = 0·897, 1·043; CI for intercept = −0·0784, 0·2826; r^2 and *P*-values correspond to Pearson's correlation test. SMA equation for a 37-species subset: $\log_{10}(\text{LDMC}_{\text{partial rehydration}}) = 0{\cdot}02071 + 1[\log_{10}(\text{LDM}_{\text{complete rehydration}})]$; CI 95 % for slope = 0·907, 1·103; CI for intercept = −0·22306, 0·26448; $r^2 = 0{\cdot}919$; $P < 0{\cdot}0001$. Slopes did not differ significantly from 1 ($P = 0{\cdot}382$, $F = 0{\cdot}779$ for 51 species; $P = 0{\cdot}997$, $F < 0{\cdot}0001$ for 37 species) and intercepts did not differ significantly from 0 ($P = 0{\cdot}261$; $T = 1{\cdot}137$ for 51 species; $P = 0{\cdot}864$, $T = 0{\cdot}172$ for 37 species). The dotted line is the 1 : 1 line.

RESULTS

Across all species, LDMC values obtained with the partial rehydration method were, on average, 4·29 % higher than, and did not significantly differ from, those obtained with the complete rehydration method (322·15 ± 15·738 and 336·58 ± 15·786 mg g^{-1}, for the complete and partial rehydration methods, respectively, $P = 0{\cdot}848$; *t*-test). Accordingly, the LDMC values obtained by the complete rehydration and the partial rehydration methods were strongly and positively correlated (Fig. 1). The slope of the relationship between the LDMC obtained by the two methods did not differ significantly from 1 and its intercept did not differ significantly from 0 (Fig. 1, legend), indicating that the two measurements were isometric. The exclusion of species with succulent and non-laminar leaves did not alter these results, either considering only the species in common with those of Vendramini *et al.* (2002) ($r^2 = 0{\cdot}919$; $P < 0{\cdot}0001$), or considering the new and more extended dataset ($r^2 = 0{\cdot}954$; $P < 0{\cdot}0001$). Note that r^2 is the coefficient of determination, which is purely a measure of goodness-of-fit and cannot be tested for significance; however, the square-root of r^2 is the correlation coefficient, which can be so tested, and this is what the *P*-values here relate to.

As expected, fully rehydrated leaves showed lower LDMC than partially rehydrated leaves of the same species, but such differences were significant in only approx. 29 % of cases (Appendix). There was no systematic difference in the relative performance of the two methods associated with any growth form: significantly lower LDMC using the complete rehydration method was observed in individual species belonging to different life forms, and there were also some cases of significantly higher LDMC (Appendix). There was no difference between LMDC estimated by the two methods among families with three or more member species (Anacardiaceae, Asteraceae, Chenopodiaceae, Fabaceae, Poaceae, Portulacaceae) ($F = 0{\cdot}31$, $P = 0{\cdot}9032$, d.f. = 5, 31; ANOVA).

Differences between methods, expressed as the absolute value of the percentage difference of LDMC values obtained with the partial rehydration method with respect to those obtained with the complete rehydration method became significantly smaller as LDMC increased ($r = -0{\cdot}517$; $P < 0{\cdot}001$). At the lower LDMC end there were plants that according to Vendramini *et al.* (2002) represent contrasting resource-use strategies: xerophytic succulents and, to a lesser degree, tender-leaved herbs typical of mesic habitats (Appendix). Among these plants, both positive and negative differences were observed between the two methods, although, because of the high within-species variability, not all of them were significant.

DISCUSSION

The present comparison of the protocols described by Garnier *et al.* (2001) and Vendramini *et al.* (2002) clearly showed that LDMC is highly conserved between these two procedures. When comparing leaf traits in datasets from different areas of the world, Vile *et al.* (2005) found that the data obtained by Vendramini *et al.* (2002) for Argentina and by Wilson *et al.* (1999) for Great Britain showed trends that differed from the rest. They put forward the lack of full leaf rehydration as the most likely cause. The possible reasons for these differences are beyond the scope of this article but, at least for the set of species of Vendramini *et al.* (2002), the present results show that they were not a consequence of the rehydration procedure. The protocol of Garnier *et al.* (2001) is in principle the best method to measure LDMC and the one recommended for situations in which it is feasible. Not only are a growing number of species around the world now been measured using it, but it also tends to allow a higher degree of rehydration, which ensures that LDMC can be used as a surrogate of leaf density (cf. Garnier and Laurent, 1994). This is particularly critical when the main focus of interest is on knowing in a precise way the absolute LDMC of species or populations. However, the procedure used by Vendramini *et al.* (2002), which does not require full rehydration and is thus less laborious, produces similar results over a wide range of leaf types. Therefore, it appears as an acceptable option for those situations in which the complete rehydration method cannot be applied. The fact that LDMC measured with the partial rehydration and the complete rehydration methods were proved to be isometric variables allows LDMC values measured with the two methods to be mixed in the same dataset when new standard measurements are not feasible.

No evidence was found suggesting that either method is more suitable to particular growth forms or families. However, the fact that the methods agreed better as LDMC increased suggests that they are most compatible in the case of sclerophyllous (= high-LDMC) leaves. The behaviour of the partial rehydration method tends to be more erratic for both succulent and tender leaves (= low LDMC), and thus it is in those cases where the greatest caution is advised. LDMC measurements carried out using the partial rehydration method on the same species and the same geographical region in different years by Vendramini *et al.* (2002) and in the present study, did not differ significantly ($P = 0{\cdot}166$, *t*-test), suggesting an overall consistency of the partial rehydration method across seasons, years and local populations. However, in about one-third of the species, the LDMC values differed by $>20\,\%$, i.e. intra-specific variation was more important than differences attributed to different rehydration protocols. A more general corollary of this is that, when comparing many studies across unrelated datasets, differences in the measurement protocol should be less important than differences among seasons, years and the quality of local habitats.

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APPENDIX

List of species selected for the measurement of leaf dry matter content (LDMC), indicating taxonomic family, leaf type and LDMC values obtained following the procedures of Garnier *et al.* (2001; complete rehydration) and Vendramini *et al.* (2002; partial rehydration). Leaf types: TL, tender-leaved; SC, sclerophyllous; SU, succulent. Symbols in brackets in column 5 indicate significant positive (+) or negative (−) differences in LDMC measured with the partial rehydration method with respect to the complete rehydration method ($P < 0{\cdot}05$; Independent Samples *t*-test). Nomenclature follows Zuloaga *et al.* (1994) and Zuloaga and Morrone (1996*a*, *b*).

Species	Family	Leaf type	LDMC, complete rehydration (mg g^{-1})	LDMC, partial rehydration (mg g^{-1})
Forbs				
Ambrosia tenuifolia	Asteraceae	TL	248	229
Eryngium agavifolium	Apiaceae	TL	206	215
Evolvulus sericeus	Convolvulaceae	TL	289	287
Trifolium repens	Fabaceae	TL	166	167
Zinnia peruviana	Asteraceae	TL	147	158
Tussock grasses				
Cortaderia rudiuscula	Poaceae	SC	396	414
Festuca tucumanica	Poaceae	SC	468	480
Pappophorum caespitosum	Poaceae	TL	365	394 (+)
Poa stuckertii	Poaceae	SC	388	371 (−)
Schizachyrium condensatum	Poaceae	TL	383	356
Trichloris crinita	Poaceae	SC	320	381 (+)
Short graminoids				
Carex fuscula	Cyperaceae	TL	335	325
Guadua trinii	Poaceae	TL	441	456
Juncus uruguensis	Juncaceae	SC	373	372
Muhlenbergia peruviana	Poaceae	TL	280	291
Neobouteloua lophostachya	Poaceae	TL	413	417
Oplismenus hirtellus	Poaceae	TL	246	257
Deciduous shrubs and trees				
Acacia aroma	Fabaceae	TL	409	421
Acacia caven	Fabaceae	TL	402	422
Brugmansia suaveolens	Solanaceae	TL	134	151 (+)
Cercidium praecox	Fabaceae	TL	339	375 (+)
Croton sarcopetalus	Euphorbiaceae	TL	243	288 (+)
Flourensia campestris	Asteraceae	TL	286	327
Geoffroea decorticans	Fabaceae	TL	347	366
Mimozyganthus carinatus	Fabaceae	TL	391	379
Prosopis flexuosa	Fabaceae	TL	365	413 (+)
Prosopis strombulifera	Fabaceae	TL	440	478 (+)
Prosopis torquata	Fabaceae	TL	420	426
Schinopsis haenkeana	Anacardiaceae	TL	474	493 (+)
Ziziphus mistol	Rhamnaceae	TL	418	412
Evergreen shrubs and trees				
Aspidosperma quebracho-blanco	Apocynaceae	SC	417	394
Schinus molle	Anacardiaceae	TL	387	363 (-)
Baccharis salicifolia	Asteraceae	TL	251	304 (+)
Capparis atamisquea	Capparaceae	SC	452	432
Fagara coco	Rutaceae	TL	263	294 (+)
Larrea divaricata	Zygophyllaceae	SC	389	466 (+)
Lithraea molleoides	Anacardiaceae	TL	425	433
Polylepis australis	Rosaceae	TL	353	359
Tricomaria usillo	Malpighiaceae	TL	224	250
Aphyllous shrubs				
Bulnesia retama	Zygophyllaceae	SC	534	608 (+)
Senna aphylla	Fabaceae	SC	398	416
Prosopis sericantha	Fabaceae	SC	515	483
Leaf succulents				
Allenrolfea patagonica	Chenopodiaceae	SU	171	170
Atriplex argentina	Chenopodiaceae	SU	250	285 (+)
Grahamia bracteata	Portulacaceae	SU	82	130
Heterostachys ritteriana	Chenopodiaceae	SU	264	270
Maytenus vitis-idaea	Celastraceae	SU	237	294 (+)
Portulaca grandiflora	Portulacaceae	SU	118	155 (+)
Suaeda divaricata	Chenopodiaceae	SU	172	193
Talinum polygaloides	Portulacaceae	SU	88	62
Bromeliads				
Bromelia urbaniana	Bromeliaceae	SU	309	282