Journal of Ecology

FORUM

Biomass–density data analysis: a comment on Cabaço *et al.* (2013)

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Summary

1. Appropriate use of mathematics and statistics is fundamental for sound interpretations of ecological results and to prevent inaccurate conclusions.

2. Throughout the article by Cabaço *et al.* (2013) emerge cases of biased data analyses including absence of statistical tests, application of unsuited tests, inconsistent geometrical interpretation of *xy* data scatter, among others.

3. These biases congregated into incorrect conclusions including (i) reporting a generalized nutrient limitation of seagrass meadows, (ii) proposing the intraspecific biomass–density relation of seaweeds as an ecological indicator, when results report little more than randomness, thus suggesting this relation is unsuited as an ecological indicator; (iii) contradicting general ecological theory without any statistical evidence; and (iv) misassociating their results to the ones by other authors.

4. *Synthesis.* In order to help ecological researchers pinpoint sources of bias, we point out mistakes related to xy data analysis in Cabaço *et al.* (2013) that can occur in any subject area and flag others specific to biomass-density relations.

Key-words: analysis of covariance, discriminant function analysis, facilitation, intercept difference, model II regression, PCA, plant population and community dynamics, self-thinning, slope difference

Introduction

The mathematical and statistical methods of data analysis in ecological studies have attained a high degree of complexity aiming to minimize the risk of deriving inaccurate conclusions. In a recent paper, Cabaço *et al.* (2013) analyse data from experimental and descriptive studies on seagrass biomass and density responses to nutrient enrichment, to (i) evaluate the intraspecific mechanisms operating within populations and (ii) determine whether biomass–density relationships can provide relevant metrics for monitoring. We contend that there are serious flaws in the data analyses and interpretation leading to incorrect or unsustained conclusions as they stand in the paper. The argumentation presented here addresses five topics: (i) general interspecific biomass–density relations, (ii) interspecific static self-thinning line, (iii) intraspecific dynamic biomass–density relations, (iv) facilitation versus competition and (v) other comments.

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General interspecific biomass–density relations

Discriminant function analysis (DFA) is the appropriate tool to synthesize multivariate data following a functional form that best discriminates observations relative to distinct categorical groups (Manly 1986). In the current case, it is a bivariate array with percentage changes in density and biomass of observations from experimental or descriptive studies (Fig. 1 in Cabaco *et al.* (2013)). We performed an optional first step in DFA measuring the Mahalanobis distance from each of the observations to the groups' centres, having found 8 of the 28 observations (28.57%) were actually closer to the centre of the opposite group (Tt^d, Tt^e and Zc^b in experimental studies and Tt^b, Tt^c, Cs, Pa and Si in descriptive studies). Having two numerical data variables and one categorical (two groups) data variable it was only possible to obtain one discriminant function of the form $z_i = 0.83x_1 + 0.56x_2$, which a $\phi_i = 1.98$ compared to a χ^2 distribution with two degrees of freedom determined non-significant with $P = 0.1411$. Therefore, experimental and descriptive studies did not differentiate from each other on the account of their alleged synchronized biomass-density responses to nutrient increases. In fact, such synchronization did not even occur. Inadequately,

Cabaço *et al.* estimated a regression line with all studies clumped together and erroneously interpreted it as a synchronized biomass–density response to nutrient increase. In fact, only because the data were well distributed between the first and third quadrants of the (x,y) scatterplot the respective regression line showed a significant positive slope. However, 'synchronized' implies a simultaneous similar response of biomass AND density, which did not occur with the general trend being a response from either biomass OR density. This biased interpretation arises from using an analysis performed over a larger data set to erroneously conclude about the specificities of subsets. A linefit specific to the descriptive studies showed such synchronization did not occur as increments in density and biomass were undoubtedly uncorrelated ($r^2 = 0.017$) and $P = 0.948$. The slope was 0.017, non-significant ($P = 0.066$) and bounded within the -0.528 and 0.562 estimated for the 95% confidence intervals. Furthermore, based in their general linefit, the authors inappropriately concluded that seagrass meadows in experimental studies were nutrient limited, although a response to increased nutrients by increasing biomass and density only occurred in 6 of 11 studies (representing only 54.5%). Then, Cabaço *et al.* justified this alleged nutrient limitation with the analysis relative to their Fig. 4, where they showed statistical evidence that a response from biomass was not followed by a similar response from density, thus denying their own 'synchronization' claim. Simultaneously, Cabaço *et al.* argue that the general regression line being above the 1:1 line demonstrates that, overall, the above-ground biomass of seagrasses responds more than density to nutrient increase. This statement would be correct if the regression line reported only to observations in the first quadrant. Nonetheless, 27.3% of the experimental studies and 57.1% of all the studies appear in the third quadrant, where the regression line above the 1:1 line means precisely the opposite. There were still six studies scattered over the second and fourth quadrants. Again, bias came from concluding about the specific dynamics of experimental studies relying on a regression where 60.7% of the observations were descriptive studies. As a consequence of an inadequate data analysis, Cabaço *et al.* produced an unclear and sometimes speculative discussion where they try to force fit ecological theory and previous results by other authors to their biased interpretation of results.

Static interspecific biomass–density relationship

There were three major flaws which had an impact on the results and conclusions:

1 Because their estimation is not independent, when inferring about differences in regression coefficients, a test on the significance of differences among slopes is the first step. Only when slopes are not significantly different does it become meaningful to test the differences among intercepts. Yet, these must be estimated using the pooled slope, the ANCOVA procedure already applied to biomass-density rela-

tionships by Arenas & Fernandez (2000). Furthermore, whenever the *y*-to-*x* correlation is not significant, and thus, neither is the regression ANOVA, the correct slope is zero. This is the analysis of covariance (ANCOVA) procedure (Sokal & Rohlf 1981; Dowdy, Wearden & Chilco 2004), which the authors did not follow (Fig. 2).

2 This point is better explained with a preliminary presentation about the geometry in (x,y) scatter analysis. Consider (x, y) data homogeneously scattered within a circumference. Whenever there is no hierarchical relation between x and y (as is the case with biomass–density relationships), the appropriate is to use model II regression (such as Principal Components Analysis (PCA)) minimizing residuals obliquely to the regression line. However, any line crossing the centre of this circumference gives the best fit. It is the well-known random rotation of the PCA axis around a population multivariate mean (Jackson 1991; Jolliffe 2002). As the line is rotated, increasing the slope, its intercept inevitably decreases, and vice versa. Slopes estimated from random sampling (particularly with small sizes) are prone to a similar bias whenever correlations are weak irrespective of the relative magnitudes of x and y variances, i.e., data need not to effectively scatter in a circumference. Testing the occurrence of this phenomenon can simply be done by estimating the bootstrapped distributions of the slopes, a technique generally well known to researchers dealing with biomass–density relations. It is the basis of the algorithm testing the overlap of the 95% confidence intervals of the bootstrapped distributions of slopes to determine the significance of their differences (Sokal & Rohlf 1981), so widely used in biomass–density studies ever since.

3 The 'high' and 'low' values close to the bottom-left corner of Fig. 2a in Cabaço *et al.* are outliers. Their placement in the normal distributions estimated for the 'high' and 'low' nutrient levels in experimental studies were $P_{\text{High, Dens}} = 0.011$, $P_{\text{High,Biom}} = 0.0076$, $P_{\text{Low,Dens}} = 0.0051$ and $P_{\text{Low,Biom}} = 0.009$; these are extraordinarily low values considering there were only 11 sampling units. These outliers must be removed from the analysis as they are enormously biasing the results.

Reanalysing the data in Fig. 2 in Cabaço *et al.*, new slopes were obtained: $s_{\text{High}} = 0.399$ and $s_{\text{Low}} = -0.363$ for experimental studies, and $s_{\text{High}} = -0.494$ and $s_{\text{Low}} = -0.326$ for the descriptive studies. These are clearly different from the previous estimates. Both permutation tests and *t*-tests determined that none of the slopes were significantly different. In particular, the difference between the experimental studies exhibited non-significances of $P = 0.344$ if using the permutation tests and $P = 0.953$ if using the *t*-tests; this is in clear contrast with the previous $P = 0.02$ presented by Cabaço *et al.* Furthermore, new tests revealed that all slopes reported to weak correlations ($r^2_{\text{Exp,Low}} = 0.0511$, $r^2_{\text{Exp,High}} = 0.1305$, $r^2_{\text{Des,Low}} = 0.0525$ and $r^2_{\text{Des,High}} = 0.0568$), and that none of the slopes were significantly different from zero ($P_{\text{Exp, Low}} = 0.5616$, $P_{\text{Exp,High}} = 0.3$, $P_{\text{Des,Low}} = 0.3399$ and $P_{\text{Des, High}} = 0.3761$). These results were a confirmation of what had

already been obtained by Cabaço *et al.* for the descriptive studies (aside from the separate issue of them presenting positive correlation coefficients for regressions with negative slopes, which is mathematically impossible), but a contradiction to their own results relative to the experimental studies due to the elimination of the two outliers. Slopes not significantly different between 'low' and 'high' nutrient levels and not significantly different from zero imply two contradictions to the discussion by Cabaço *et al.*: (i) they cannot sustain any hypothesis about nutrient limitations, and (ii) they do not reflect two opposite responses corresponding to the study type. In fact, these slopes only reflect random PCA axis rotations pivoting the bivariate means of the samples as demonstrated by the bootstrapped distributions of slopes with the 'Exp-High', 'Exp-Low', 'Des-High' and 'Des-Low' exhibiting maxima of 303, 403, 683 and 333, and minima of -383, -1017, -799 and -156, respectively. Also Cabaço *et al.* estimated the bootstrapped distributions of the slopes to perform the analysis in their Fig. 2, having overlooked this valuable hint. Then, we proceeded to a test upon the new intercepts: we set the slopes to zero and estimated the intercepts, which represent the expected seagrass biomass irrespective of species and density. Their estimated values were $a_{Exp,High} = 1.968$, $a_{Exp,Low} = 1.791$, $a_{Des,High} = 1.854$ and $a_{Des,Low} = 2.118$. The permutation tests determined that none of the intercepts were significantly different from each other. In particular, $P_{Exp} = 0.189$ and $P_{Des} = 0.069$, whereas the *t*-tests determined that within each study type 'low' and 'high' intercepts were always significantly different ($P_{Des} = 3.13 \times 10^{-9}$ and $P_{Exp} = 2.5 \times 10^{-4}$). The reasons for the divergence of results yielded by *t*-tests and permutation tests would be a separate discussion of a statistical nature which is beyond the scope of this comment. We believe divergence may be due to the assumption of normality required by *t*-tests. Thus, we prefer to rely on the permutation tests, which by stating expected maximum seagrass biomass being similar under low and high nutrient levels, refutes the nutrient limitation of experimental studies proposed by Cabaço *et al.* Nevertheless, if one prefers to follow the *t*-tests and accepts the differences as significant, then the intercepts support the claims by Cabaço *et al.* of two opposite responses to nutrient limitation according to the study type as a consequence of the time-scales of the processes involved. But then, the present analysis is the correct procedure demonstrating the different expected seagrass biomasses under the specified conditions. This interpretation is in accordance with the posterior analysis Cabaço *et al.* did in their Fig. 4. and contrasting with their fit of a non-significant difference between slopes arising from the random rotation of PCA axes derived from weakly correlated data sets (in their Fig. 2).

Dynamic intraspecific biomass–density relationship

Intraspecific self-thinning has a typical dynamic that is conspicuous in log*B*-to-log*D* plots. Consider two monospecific stands of the same species, both undergoing active growth,

nevertheless subject to a difference in resource availability and therefore in competitive stress. Both start with the highest densities and lowest biomasses at the bottom-right side of the log*B*-to-log*D* plot. In time, both progress towards the top-left side of the plot (Creed 1995; Morris 1996; Arenas & Fernandes 2000). The stand subjected with stronger competition exhibits smaller biomass increments associated with bigger mortality losses, resulting in a flatter negative slope and a lower intercept. The stand subjected to weaker competition, on the other hand, exhibits bigger biomass increments associated with lesser mortality losses, resulting in a steeper negative slope and a higher intercept. Such were the results obtained by Morris & Myerscough (1991), Creed, Kain & Norton (1998), Rincón & Lobón-Cerviá (2002), Morris (2003), Steen & Scrosati (2004) and Vieira & Creed (2013a, b). Their regression coefficients are not comparable to those in Fig. 3 by Cabaço *et al.*, where all slopes having positive estimates demonstrated that modular construction of seagrasses offset self-thinning. Furthermore, Cabaço *et al.* argued that high nutrient levels always showed steeper slopes, but in one of nine cases, the slope was actually flatter (Tt[d]), and in five cases, the differences were too slim to attempt such a statement (Cn, Ho, Th[b], Tt[f] and Zm[c]). Then, Cabaço *et al.* generalized about a conspicuous association of steeper slopes with lower intercepts. However, almost all possible slope × intercept combinations occurred in the nine plots. Under these circumstances, it is essential to test the significance of differences between slopes and between intercepts, as was previously shown for the interspecific biomass–density relations. Unfortunately, we cannot perform the reanalysis as it is not possible to accurately retrieve the data from Fig. 3. Nevertheless, we consider it reasonable and legitimate to question whether any significant differences did occur. The dispersion clouds in Fig. 3 match the weak correlations and approximate *x* and *y* variances for which PCA is less reliable, accordingly to Jackson (1991). As in the previous section, these regressions may easily have resulted from random PCA axis rotations around the bivariate means of samples randomly collected from a common population. This is a much simpler and more logical hypothesis than the one presented by Cabaço *et al.*, which is intricate, speculative and absent of any statistical confirmation. Therefore, neither may their conclusions be associated with the ones by Morris (2003), Steen & Scrosati (2005) and Chu *et al.* (2010), nor may this alleged pattern be proposed as an ecological indicator.

Competition versus facilitation

According to Chu *et al.* (2010), all predicted plant stand trajectories exhibit negative slopes as a consequence of self-thinning induced by competition, whereas facilitation affects the steepness of the negative slopes. There is no correspondence between the steepness of these negative slopes, and the steepness of the positive slopes obtained for the non-thinning stands in Cabaço *et al.* In previous work by Chu *et al.* (2008), the positive slopes were indeed observed evidencing facilitation. Yet, these were only in the lower density phase

of the population trajectory on the left side of the log(*b*)–log (*D*) plot, whereas the right side exhibited the traditional negative slopes. The hump these trajectories exhibited, with maximum biomass at intermediate densities, was not even remotely present in the plots by Cabaço *et al.* Nevertheless, Cabaço *et al.* present the 'high' and 'low' nutrient regressions of *Zostera noltii* intercepting close to the average density as evidence that 'shoot biomass decreases with density at densities lower than 8511, whereas it increases above that threshold ... This is the only species where the density threshold is in the middle of the density distribution range'. Such inference can hardly be achieved and it is intriguing how such conclusions were drawn and interpreted as evidence of a facilitation threshold. Taking on a different perspective, this is most probably the random PCA axis rotation pivoting the statistical population bivariate mean already discussed above.

Other comments

Cabaço *et al.* argued 'high' nutrient levels (more stressed populations) exhibited lower biomass and density coefficients of variance than 'low' nutrient levels (less stressed populations) without presenting any statistical evidence supporting this claim (Table 3 and page 1559 last paragraph). In fact, throughout the section devoted to interspecific biomass–density relations, the authors never performed any statistical tests that could eventually corroborate their findings. Looking at the dispersion clouds, one may reasonably wonder how these could report statistically significant variances. Strong statistical proofs would have been essential before contradicting the general trend of ecological responses to disturbance-driven changes (e.g. Sousa 1984; Underwood 1992; Turner 2010).

Cabaço *et al.* performed bootstrap sampling with an astonishingly low 50 replicates, which is inacceptable given the current computation capabilities. All randomization tests performed in this comment used 10 000 replicates, with each test taking no more than 3 s to compute. Also, it is insufficient to extract only the standard error from bootstrapping, when it provides full empirical distributions including all desired confidence limits. However, with only 50 replicates, the distributions can only be defined with 2% precision, implying there is not even a 5% confidence limit as distributions jump from 6% to 4%. With 10 000 replicates, the distributions were defined with 0.01% resolution, implying the 5% confidence limit region progressed from 4.99% to 5% to 5.01%.

Data accessibility

The data used for re-analysis in this article was retrieved by directly measuring the data from screen-magnified Figs 1 and 2 in Cabaco *et al.* (2013).

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