

Journal of Ecology to matthew.barbour, admin

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Partitioning plant genetic and environmental drivers of above and belowground community assembly
JEcol-2017-0556

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Dear Dr. Barbour,

Thank you for submitting this paper to Journal of Ecology. I have now received referees reports, copies of which are included below. I have also discussed the paper with Ignasi Bartomeus, the Associate Editor responsible, whose assessment is below, and with Mark Rees, one of Journal of Ecology's Editors.

As you can see, while the referees and Editors are positive about the study and feel the work presented here has considerable potential, they also express significant doubts about the current presentation. To address these comments fully would result in a much-altered paper and additional outside review will be needed. In these circumstances it is our policy to reject the current version of the manuscript but to invite resubmission to Journal of Ecology once you have resolved the problems identified.

We hope that you will consider resubmitting a new version of this work to Journal of Ecology and would be grateful if you will let us know whether you intend to do so. If you do wish to re-submit, you should do this using our website within four months of the above date. Please be aware that our policy is to treat resubmissions as new manuscripts in the sense that they will go through a further stage of peer review, possibly by the same reviewers as used for the original submission, and that there is no commitment from us as to eventual acceptability. I am sorry that I am unable to be more positive at this stage, but we hope that you can use the comments and advice given below to produce an improved submission that may reach an acceptable standard.

Once you have revised your manuscript, go to <http://mc.manuscriptcentral.com/jecol-besjournals> and login to your Author Center. Click on "Manuscripts with Decisions" and click on "Create a Resubmission" located next to the manuscript number, and then follow the steps for resubmitting your manuscript. Please upload a word processor file, which will be converted to a pdf by the system, rather than creating the pdf file yourself.

Please include with your resubmission an itemised list detailing how you have responded to each of the various comments on this version, plus any other supporting statements you would like us to consider.

Please do not hesitate to contact me should you require any further assistance or information. All correspondence regarding the decision should be sent to the editorial office, rather than direct to the editors or reviewers.

Best wishes,

James Ross
Assistant Editor

Associate Editor

Handling Editor Comments for Authors:

I concur with the 3 reviewers that the paper presents a very nice experiment, but that the results are hard to integrate. There are several points that need major special attention including the use of Type III ANOVA when you test interactions (R1), the effectiveness

of the aphid treatment, given its low numbers (R2, but also mentioned by others) and the clarity and integration of the paper (R2 & 3). The nature of the critics raised needed exceeds those of a typical "major revisions". However, I would like to leave the door open to resubmitting a new paper with the same data if the authors are willing to substantially re-write the ms to incorporate reviewers suggestions and make a more focused paper.

Referees' comments:

Reviewer: 1

COMMENTS FOR THE AUTHOR

I have read the manuscript 'Partitioning plant genetic and environmental drivers of above and belowground community assembly'. It reads well and includes a lot of detail on the complex interactions that we see in natural communities. Certainly, we are missing studies on the relative importance of genetic and environmental factors. However, I feel that the final message is somewhat that the traits you studied elsewhere in a different study (plant chemical variation) are likely the ones that explain more variation than those you measured here (mostly plant growth traits). By using the older data as a proxy you make the case for this (and I am aware that often not everything can be analysed), but it does leave the message of this paper missing the final link.

Major comments

1. I would move the hypotheses hidden in the methods 'study site' section to the end of the introduction, probably after the main research questions. Here you would also have to extend the introduction to include the background of the system, i.e. the section in the methods before the hypotheses.
2. In the experimental setup, (L164) were aphids added at each new time point (this is how I understand it), and were then aphids removed from aphid-absent plants (if there were any)? Then if there were only 0.05-7 aphids on average on plants, does this mean there was little reproduction? How often were aphids replenished? I wonder if aphids didn't 'accept' certain genotypes, how might this impact the power of the aphid environment analyses?
3. For the analyses, the use of type II SS is ok for unbalanced data sets but I think not so suitable if there are significant interactions in the model, since it is more focused on the order (or non-order) of main effects. Please can you justify the use of these, and do the models differ when you use type III SS? i.e. how robust is the data to the different types.
4. There is some inconsistency in the interpretation of the wind experiment, with respect to the relative effect of genotype and environment. For example, in the results (L492) you say the abiotic is more important yet later at L632 you say that genotype is more important in both, then L658 again you say the environment dominated. I think this partially just needs a clarification in the text on what you are looking at, since wind has perhaps a stronger effect on plant traits, height and shoot length (but not shoot count), yet less effect on foliar arthropods, for richness and abundance (but not rarefied richness). Yet, the abstract again suggests that wind is more important than genotype for foliar arthropods and bacteria. Please clarify in the text at each stage again what it is that you are discussing.
5. In the discussion, from L672 and the next few paragraphs, you mention a couple of times 'the only other genotype-by-environment experiment/study.' but then have multiple citations across the text. The importance of each of these studies in relation to yours and others needs to be clearer, what is unique to that study that has not been done before, and then how is your progressing from these.
6. The aboveground and belowground communities have been studied rather independently. I would expect feedback from each, as plants can change their rhizosphere community in response to herbivore feeding. Also, aphids can respond to changes in the rhizobacteria/fungal community belowground. How might feedback from these communities have effected one another? I'd like to see some discussion on this.

Minor comments

1. Figure 1A needs explanation somewhere of the black and white dots, I assumed this to be aphid +/- treatments.
2. Figure 2A, can you give a bit more explanation (maybe in the text at L486), on what this is actually showing and how this demonstrates the interaction is driven by genotype J.
3. Figure 4. What do you mean the marginally significant effects are transparent? Clarify this.
4. Methods L261 and 264, add in that you also included all 2-way interactions to make the methods fully clear

5. Results. L419, just re-mention which species name is the ant and which is the aphid (I had to go back to look after reading through the methods and then starting the results section)
6. Results. L 516, change Fig. 1C to Fig. 2C, and L524 Fig. 1D to Fig. 2D
7. Results. L519, Table A6, could you add the functional group of each OTU/species to the table?
8. Results. L570. Change Table 11 to Table 1
9. Discussion. L714. Compared to what other studies? Give some citations here.

Reviewer: 2

COMMENTS FOR THE AUTHOR

I like that the authors manipulated two sources of environmental variation (wind, aphids) and replicated this at multiple sites. These aspects make the study very complete. Here are a few general comments and then more specific ones for each section.

1. I would suggest reducing/simplifying the statistical analyses and the results section is too long. Perhaps you could focus only on effects on the associate community and then the SEM to tease a part genetic and direct and indirect effects of the environment. Maybe the section on treatment effects on traits could be reduced substantially or maybe left as part of the methods (referring to supplementary material for these results). Could also delete some analyses (e.g. wind effects on soil characteristics) that did not yield important findings. At this moment, it is difficult to grasp the main findings of the results section so I recommend simplifying and focusing on the core analyses and key results.
2. The effect of site could be viewed as another source of environmental effects on plants and their associated faunas. Perhaps the relationship the site effect and the treatment effects could be addressed up front. The design clearly has advantages in this sense, and maybe these should be explicitly stated and capitalized on, i.e. the relationship between treatment and site effects as environmental influences on plant associates.
3. Can direct vs. indirect effects of the environment be effectively separated based on the current design and analyses? Does the lack of plant trait effects mean that direct effects must be acting or that residual variation not explained by traits are necessarily due to direct effects of the environment? Or is it that other unmeasured plant traits could be driving effects on the community but were not evaluated (i.e. indirect effects were not adequately tested)? I can't see how one could determine one scenario vs. the other, so perhaps some more thought has to be put into the a priori expectations of what is being tested as well as the interpretation of the results.
4. I did not understand how these SEM models were fed (see specifics further down). This is a key part of the study which relates plant genetics, plant traits, and environment to the associate community and I would like to know more about the basics of this analysis. See some specifics on this ahead.

*Side comment: the lead author has used the term community "assembly" in a previous study, but I wonder if this could be a bit misleading since assembly dynamics, in the strict sense, were not addressed. Maybe this is just a matter of opinion and a more loose use of this term is fine. For example, I was imagining a different terminology, such as "effects on community structure", "structuring of associate communities", etc. that didn't imply assembly dynamics per se (stuff like priority effects and other temporal dynamics of community properties).

Introduction

This section emphasizes the importance of addressing connections and feedbacks between above- and below-ground communities associated with plants. However, the paper ends up delivering an isolated assessment of effects on each component. I wouldn't necessarily suggest adding more analyses to explore these connections (the paper is already heavy on the analytical side), but still, this part of the intro could generate some expectation that these dynamics will be evaluated. Perhaps just modify the language a bit to tailor to limits of the present work. Also, perhaps the term "phenotypic plasticity" could be narrowed down to "plant-mediated effects on associated faunas" (here and throughout the paper). This is a bit more specific and relates to plant induced responses influencing associates, and you could relate this parenthetically as a form of plasticity, to connect to a broader context.

L. 43-46: Direct and indirect effects can be (and have been) evaluated in controlled experiments, I think the issue is that they're conducted in a single site. The key point is that having site replication (e.g. ideally along an environmental gradient) is important in order to address the context dependency or spatial variation in such direct/indirect dynamics.

L. 92: H estimates are environment-dependent, so this begets to some extent the logic of this sentence. Maybe talk more broadly about magnitude or degree of genetic variation varying among traits?

L. 95: Wording for q. 1 is not clear to me, particularly the ending "... and other environmental factors". Also, might be good to specify in q. 3 that this refers to plant genetic variation and plant plasticity as bottom-up sources of variation in associated faunas.

Methods

What was the spatial scale (area over which gardens were distributed) for the aphid and wind experiments?

L. 126-29: The distinction between these three alternative dynamics don't seem clear cut. For example, aphids may directly influence other herbivores or indirectly do so via changes in plant traits. Perhaps divide these dynamics into direct (e.g. competitive) and indirect (plant-mediated or ant-mediated) effects of aphids on the associate community. Also, might be helpful to relate this back to biotic effects of the environment (rather than genetics) on plant phenotypes.

L. 144-46: Again, perhaps thinking about direct and indirect effects of this environmental source on plant-associated communities (or some other division) is a cleaner (and simpler) way of thinking of pathways of potential effects (and within each of these two categories provide examples such as those described).

L. 222-224: It seems somewhat problematic that phenolic compounds were not quantified for the actual plants used in the experiment. Heritability values are environment-dependent this justification may be weak. Particularly the last line "and therefore unlikely to be strongly influenced by environmental variation" is questionable since phenolic compounds (and other groups) are highly inducible (e.g. by abiotic factors such as light availability, not only herbivory). As the authors mention, there's also the potential for G x site and G x treatment effects that are not addressed by using a different set of plants to quantify defenses. I'm not sure if these limitations defeat the purpose of including phenolics in the paper, particularly given that genetic and environmental sources of plant phenotypic variation are the specific subject under study.

L. 260: Any rationale/justification for including genotype (and its interaction) as a fixed rather than random effect?

L. 263-64: was this a repeated-measures GLMM?

L. 273: it is not clear from this description that the analysis of wind effects was based on a split-plot design. Was the wind x G interaction assessed? This model structure appears different than the model for the aphid treatment but shouldn't they be the same (except for the year effect)?

I. 358: So these models included significant in the models above, where they were treated as fixed effects? This is confusing to me, both using subsets of significant terms and re-running models (sort of like a non-declared stepwise procedure) as well as treating factors as fixed in one analysis and then as random in another. Are these analyses somewhat redundant relative to the SEM analyses in the next section. Both seem to be quantifying the relative importance of genetic and environmental (plant-based and non plant-based) variation.

L. 359-61: Can you separate direct from plant-based (indirect) environmental effects in this analysis?

L. 369: This section is key, but I was not able to understand how genetic and environmental effects were quantified (is this related to the variance components estimated from analyses described in the previous paragraph?). I would like to know what specific data on genetic and environmental effects were inputted in the SEMs. This section provides technical information on the statistical features of the analysis, but I can't understand the basics of how the effects were estimated and the type of data that was fed to these models.

Results

L. 424-25: Were aphid abundances kept roughly the same among plants in the aphid treatment? Five aphids were inoculated per plant, and based on the genotype means for aphid abundance (0.05 to 7 aphids) it seems like aphid numbers later in the experiment were similar to the number initially inoculated. If this is the case, how can genetic variation in ant abundance (mediated by aphids) be evaluated within the aphid treatment if aphid numbers were relatively constant among plants and genotypes and similar to initial numbers?

L. 492: were there G x wind effects on abundance, richness?

Reviewer: 3

COMMENTS FOR THE AUTHOR

Barbour et al. present a comprehensive study on the partitioning of variation found among willow genotypes. Using two different large field experiments, the authors test how a number of variables affect the arthropod community on willows aboveground, and the community of microorganisms belowground. The variables of interest include plant genotype, variation in several plant traits, presence of a keystone herbivore (aphids), and an abiotic condition (wind exposure). I am very impressed by the size and quality of the study, and in my opinion each experiment represents the state-of-the-art for field experiments on variation partitioning. Unfortunately, while each experiment is strong individually, the combination of the two the main weakness of this study in my opinion.

The two experiments use the same set of plant genotypes, are carried out in the same geographic area at the same time, and use largely the same statistical toolset. However, there is relatively little integration of the results from the two experiments. As an example, plant traits of the willow genotypes are measured in both experiments, but are never directly compared between experiments. Among the few places where results are compared side-by-side are table 1 and figure 1a-d, but these comparisons raise more questions than they answer. Table 1 presumably is meant to compare relative contributions of genetic and environmental effects in the two experiments, but since most values are missing for the ant-aphid experiment the only meaningful comparison is for the two values of arthropod abundance. It is therefore unclear what message the authors want to convey with this table. Additionally, it highlights that the two experiments were carried out in different years, raising the question whether these (presumably unitless?) net effect sizes can still be compared, and how large the year-to-year variation would be within experiment. Are the values in Figure 1 from different years as

well, or were they all measured in the same year? Are the values in panels a-b and c-d meant to be compared directly? If so, the y-axes should be on the same scale.

While the two experiments share many similarities, there are important differences, including total duration (years with data) and traits measured in different years. The two experiments are presented sequentially in each section of the manuscript, and the authors attempt to maintain a logical structure by the frequent use of subheadings. Nonetheless, I often found this back-to-back presentation confusing, especially since the important differences between experiments are generally only mentioned once in the methods. It would be helpful if they were mentioned again when a new result is presented for which these differences are important. In addition, and given the lack of integration of the two parts, the sequential presentation often feels like a listing of results and does not make for very attractive reading.

Overall, the sheer number of information contained in these experiments makes it difficult to follow the author's arguments, and it doesn't help that the manuscript currently reads more like two stories that have been spliced together. In the current state I doubt whether the combination of the two experiments provides a benefit beyond the sum of the manuscript's parts. Given the high quality of the two experiments, it would perhaps be justified to split this story into two, and submit two streamlined manuscripts for back-to-back publication. However, if the authors decide against this approach I would strongly encourage them to attempt to integrate the results more, starting with the points that I criticized above. In that case I think it should also be possible to significantly shorten the manuscript, particularly by taking better advantage of the similarities between the experiments. In several places a section in the methods or results for the ant-aphid experiment is followed by a very similar section on the wind experiment. By reducing some of the subdivisions and condensing similar parts where possible, I think redundancies could be significantly reduced, and the manuscript be made easier to follow throughout. I acknowledge that this will not be an easy task, but one way or another simplification and streamlining of the manuscript will be necessary for it to be read by a broad audience.

In addition to this major issue I only have a few more minor comments that I list below.

L164: Was there any colonization by aphids of plants in the control plants? If so, how were these plants treated? The authors claim later in the manuscript that aphid effect on arthropod communities were independent of aphid numbers (L588), suggesting some induced change in the plant triggered by even few aphids. Presumably this could present a problem if controls were colonized by aphids even for short amounts of time?

L165: The timings of the two experiments are quite complicated. Presumably all data for the ant-aphid experiment is from 2012, but for the wind experiment it isn't always clear if data is from 2012, 2013, or averaged across years. Personally, I would appreciate a supplementary figure detailing the timings of the experiments in graphical form.

L203: I believe the denominator of the equation should be the 'fresh mass' (the higher weight) for the result to be a fraction.

L420/426: Language in which results are reported should reflect statistical significance. 'Had little effect' with a p-value of 0.46, 'had no effect' with a p-value of 0.195 is counterintuitive.

L425: I wonder if average numbers are useful for quantification of ant visits, as this depends on all censuses across which numbers are averaged to be identical in all conditions – assuming for example that ant activity followed diurnal patterns but plants were sampled randomly throughout the day, zero values could be meaningless. I'm not sure if it is a better solution, but I would at least try to compare results with the sum of observed ants as well.

L429/L449: 'would be mediated by plant traits' seems incorrect, as 'mediate' implies that plant traits are a separate actor, rather than one aspect of plant genetic variation. 'would be caused' or 'would be due to' would be better. Check usage of 'mediate' throughout.

L438: This is a surprisingly strong effect of aphid induction on trichomes, which aren't usually a very plastic trait. I think this could be highlighted more in the discussion, and I would be interested to learn the authors interpretation of this result.

L451-461: Two sets of results are presented for the wind experiment, the second set described as the end of the experiment. It is unclear to me what these time points correspond to, and this should be explained better (see also my comment regarding the unclear timings in this experiment).

L474-475: The genetic units of willow are sometimes referred to as genotypes, and sometimes as clones. For consistency and to avoid confusion the same name should be used throughout.

L570-577: The reporting of the PCA results here is very repetitive and could be significantly condensed.

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