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Submitted PDF (with line numbers)

MELNHE Website
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Username: melnhe
Password: Sh0e22$tr1ng

Thank you for this second opportunity to revise our manuscript for publication in Ecosystems. Our responses to the Editor are detailed below in blue. Excerpts from the manuscript are red Times New Roman.

Date:21-Jan-2024

Dr. Yanai, Ruth

RE: Nitrogen and phosphorus addition affect soil respiration in northern hardwood forests

Dear Dr. Yanai:

Your revised paper was evaluated by Dr. Erin Berryman, the subject-matter editor, who has recommended that minor revision will be required before the paper can be accepted for publication. I have reviewed the recommendation and agree with Dr. Berryman. A revision that addresses these final concerns will be re-evaluated by the editor before I make a final decision. The points below are should be straightforward to address.

The comments from the subject-matter editor are attached here. You may also view the information in your AUTHOR CENTER of the ECOSYSTEMS MANUSCRIPT CENTRAL site (https://nam04.safelinks.protection.outlook.com/?url=https%3A%2F%2Fmc.manuscriptcentral.com%2Feco&data=05%7C02%7Cforestecology%40esf.edu%7C7798927b01ef4e2bb73908dc1ac1adc9%7C471cf45e787c42bda95ce748123126f6%7C0%7C0%7C638414666368442234%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=V12CXwXu9RC%2BhPpmDJRWkGL0YnB1IH2Hppc%2BUO0bvzk%3D&reserved=0).

Please highlight the major edits in your revised manuscript text. This makes it easier for reviewers and editors to view your changes within the text.

When you are ready to submit your revision, return to your AUTHOR CENTER and click the Manuscripts with Decision category in the left-hand list. Then under Actions (on right), click Create a Revision. Follow the onscreen directions to respond to Subject-Matter Editor and Reviewer comments. In the text boxes provided, enter (type or copy and paste) your cover letter explaining, point-by-point, the changes (and text location) you have made in response to each reviewer or editor comment. Remember to save a copy of the letter for your records. Next, complete manuscript details, and upload revised files. Remember that after you have viewed your PDF and HTML proofs you MUST click SUBMIT in the lower right corner to complete the submission.

Your revision is due within 6 weeks of receipt of this letter. Revised materials received more than 3 months from the date of this letter will be regarded as a new manuscript.

Thank you for submitting your work to Ecosystems.

Sincerely,

Monica Turner

Co-Editor-in-Chief

SUBJECT-MATTER EDITOR'S COMMENTS
Subject-Matter Editor: Berryman, Erin

Comments to the Author:

The revised manuscript is improved, and I thank the authors for taking the time and care to address most of the reviewers' concerns. However, both Reviewer 1 and I highlighted the need for improvement in the Discussion, requiring a revisit of the original hypotheses and a stronger take-home message. Your response to Reviewer 1, item 3, only addresses the Introduction and ignores the Discussion.

We added to the Discussion, when addressing the effect of N: "Most studies suggest that suppression of heterotrophic respiration by added N is the primary explanation. For example, Burton et al. (2004) indicated that N suppression of soil respiration in sugar maple forests was not caused by reduced root respiration, as root biomass, turnover and specific root respiration rate were unaffected by the treatments." Regarding a reduction in microbial biomass or diversity in N-fertilized plots, we added: "In a global meta-analysis, Treseder (2008) noted a roughly 15% reduction in microbial biomass under N addition, and this biomass response was correlated with soil respiration responses. Increased N availability may impede lignolytic enzyme activity (Carreiro et al. 2000; Janssens et al. 2010), especially in high lignin detritus (Knorr et al. 2005)." For the lack of an effect of P on soil respiration, we added: "Similarly, two recent meta-analyses (Feng and Zhu 2019; Zheng et al. 2023) concluded that soil respiration is not significantly affected by P addition in temperate forests. Notably, although forest production components typically increase with addition of N and P together, no significant response of soil respiration to combined NP addition was observed for a global meta-analysis (Zheng et al. 2023). In contrast, P addition stimulated soil respiration in tropical forests, possibly reflecting very low soil P availability (Feng and Zhu 2019)."

In the section on the relationship of roots to soil respiration, we added, "Increases in growth with N or N+P addition would be expected to result in increased soil respiration, but we detected a suppressive effect of N on respiration. Thus, neither fine root biomass nor fine root growth explains the suppressive effect of N on soil respiration."

In the section on *"Temporal effects"* we added: "We expected transient changes and seasonal differences in the response of soil respiration to nutrient addition as various components of the process adjust to changes in nutrient availability (Bowden et al. 2004; Zheng et al. 2022). The N suppression of soil respiration by N was detected primarily in the summer season when soil temperature is highest and fluxes are greatest. This N suppression was consistent throughout most of the study period." Finally, the section on the mysterious increase in soil respiration over time now begins, "We did not anticipate any long-term trend in soil respiration, and…"

In addition, the hypotheses that you added in the revised version appear to contradict each other - in one, you state that no effect of P is anticipated, and in the last hypothesis, you imply that you do expect an effect of P. It may be that you just need to clarify that you expect no MAIN effect of P, but that you expect an interaction effect.

We clarified the hypotheses: "We expected a reduction in soil respiration due to N addition, as has been observed in similar experiments (Burton et al. 2004), but not due to P addition, in keeping with a recent meta-analysis of forest studies (Zheng et al. 2023)." We decided not to bring up the interaction effect.

Finally - I appreciate the addition of Figure 1, but the tree species codes need to be spelled out somewhere for reference - many people will not be familiar with USFS abbreviations.

We added the species names: "Tree species are represented using USFS species codes: ACRU = *Acer rubrum*, ACSA3 = *A. saccharum,* BEAL2 = *B. alleghaniensis*, BEPA = *Betula papyrifera*, FAGR = *Fagus grandifolia*, FRAM = *Fraxinus americana,* POGR4 = *Populus grandidentata*, POTR5 = *P. tremuloides*, PRPE2 = *Prunus pensylvanica*." We deleted the species composition information from Table 1.

We believe that these revisions have improved the paper and we hope that you will find the manuscript to be acceptable for publication. We look forward to hearing from you soon.

—----------- response to first round of reviews is below —-----------------

Thank you for the opportunity to improve our manuscript for publication in Ecosystems. Our responses to the reviewer comments are detailed below in blue. Excerpts from the manuscript are black Times New Roman with additions in red ~~and deletions indicated by a strikethrough.~~

We believe that these revisions have improved the paper and we hope that you will find the manuscript to be acceptable for publication. We look forward to hearing from you soon.

Date:01-Oct-2023

RE: Nitrogen and phosphorus addition affect soil respiration in northern hardwood forests

Dear Dr. Yanai:

The reviewers have completed their evaluation of your paper and Dr. Erin Berryman, the subject-matter editor, has recommended that major revision will be required before the paper can be considered further for publication. I have reviewed the recommendation and agree with Dr. Berryman. A major revision that addresses the substantive concerns of the subject-matter editor and the reviewers will be re-evaluated by the editor before I make a final decision. The reviewers may also be asked to evaluate your revision.

The comments from the reviewers and the subject-matter editor are attached here; all have provided thoughtful feedback. You may also view the information in your AUTHOR CENTER of the ECOSYSTEMS MANUSCRIPT CENTRAL site (https://nam04.safelinks.protection.outlook.com/?url=https%3A%2F%2Fmc.manuscriptcentral.com%2Feco&data=05%7C01%7Cforestecology%40esf.edu%7C964ba01101aa4803389908dbc2a0f346%7C471cf45e787c42bda95ce748123126f6%7C0%7C0%7C638317768777066330%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=nyIU7RMpEO7cPBfflpLOxGGxmv8WpZ6h0SVPeGRuVPI%3D&reserved=0).

Please highlight the major edits in your revised manuscript text. This makes it easier for reviewers and editors to view your changes within the text.

We are supplying both a clean version and a tracked-changes version.

When you are ready to submit your revision, return to your AUTHOR CENTER and click the Manuscripts with Decision category in the left-hand list. Then under Actions (on right), click Create a Revision. Follow the onscreen directions to respond to Subject-Matter Editor and Reviewer comments. In the text boxes provided, enter (type or copy and paste) your cover letter explaining, point-by-point, the changes (and text location) you have made in response to each reviewer or editor comment. Remember to save a copy of the letter for your records. Next, complete manuscript details, and upload revised files. Remember that after you have viewed your PDF and HTML proofs you MUST click SUBMIT in the lower right corner to complete the submission.

Your revision is due within 6 weeks of receipt of this letter. Revised materials received more than 3 months from the date of this letter will be regarded as a new manuscript.

Thank you for submitting your work to Ecosystems.

Sincerely,

Monica Turner
Co-Editor-in-Chief

SUBJECT-MATTER EDITOR'S COMMENTS

Subject-Matter Editor: Berryman, Erin

Comments to the Author:

This is a well-written report on an important topic that doesn't often get attention - long-term impacts of fertilization on soil respiration. However, it needs some work before it will be ready for publication in Ecosystems. There are some inconsistencies throughout the manuscript that need resolved, especially in linking the methods, results and discussion together. Reviewer 1 has some good ideas on how to do this. Some of the methods need to be revisited - note Reviewer 2's concern about the use of root biomass as a co-variate. Finally, both reviewers had questions about the statistical and analytical choices for assessing impacts on respiration - that should be addressed.

Our changes are detailed below. The reviewers identified some problems and suggested important improvements to the paper. We clarified the limitations of the data set in the paper, when it was not possible to adopt their suggestions.

REVIEWERS' COMMENTS

Reviewer: 1

Comments to the Author(s)

This study examines the effect of long-term nitrogen and phosphorus fertilization on soil respiration across three groups of stand ages in a northern hardwood forests. This is the longest running nitrogen by phosphorus fertilization experiment in temperate forest, and soil respiration has been measured for ten years. Mann et al. update their study with ten years of soil respiration data (from Bae et al., 2015 and Kang et al., 2016) across the 13 stands and four treatments, with 5-7 collars per plot. Root biomass was measured in 10 cores per plot. They hypothesized that soil respiration would be driven by forest dynamics, correlated to root biomass and highest in old stands, and most responsive to nutrient additions in the youngest stands. They found that nitrogen additions alone suppressed soil respiration in five of the 11 analysis periods, by about 13-14%, and that soil respiration was correlated to fine root biomass, which was lowest in the mid-successional stands.

Overall, this is an important follow-up study investigating long-term impacts of fertilization on soil respiration. I have a couple of comments:

1. Some data might need to be included in a Supplement to rationalize and justify some of the statistics. First, it is unclear when certain p-values reflect the anova results, or contrasts between the control and a specific treatment.

Our intention was to annotate the p values to indicate what they mean, like "($p = 0.01$ for the main effect of N)" We had not done this consistently, and 8 cases have been clarified. A specific example was pointed out by this reviewer on line 33, below.

Second, how common is it to use a median value to obtain a respiration value per plot? How well does the median actually reflect the data (perhaps in the Supplement, show a distribution of the collar data and then the median values)? It doesn't appear that Bae et al., 2015 and Kang et al., 2016 used medians.

We used medians because mean soil respiration values were subject to undue influence from outliers, especially in the positive direction. We found medians to be more robust against that influence. The following is our justification in the manuscript: "The response variable was generated at the plot scale by first taking the median soil respiration rate for each measurement date because the mean was sensitive to the presence of outliers."

2. Are the three month/three year groupings the best way to show and analyze the data? For example, soil respiration responses to nutrients could be driven by moisture effects that vary year to year, but the data is grouped into three-year analysis periods across three seasons. Third, soil respiration rates typically increase into the growing season, meaning that grouping March-May together, June-August together, and August-November together might not be the most ecologically meaningful. If you are most interested in the treatment and stand age effects, I would think a mixed-effects model with sampling years and months/dates could be treated as random effects, since you aren't interested in that seasonal variation. If you are interested in the interaction between nutrients and season, then perhaps grouping by season is necessary.

We added to the Data Analysis to better justify our approach: "Grouping years provided enough data to distinguish responses within seasons and over time, as the number of measurements in any given year and season was low. We consistently collected data in the summer, but due to the availability of personnel and access to study sites, spring and fall measurements were collected less frequently (Appendix A)."

We had not described our interest in seasonality, but we added to the Introduction: "On top of these developmental factors, seasonal changes in microbial communities (Sorensen et al., 2018) and rates of root growth (Abramoff & Finzi, 2016) could also be expected to affect soil respiration." and to the hypotheses: "Finally, we predicted that the effect of N and P addition on soil respiration would vary by season due to seasonal changes in root growth and soil microbial communities."

Regardless, it may be worth showing a cumulative flux by year or multiple years to get at the overall nutrient effects (see Bowden et al. 2004 and Taylor et al., 2021).

Because there was only one year post-treatment in which we measured soil respiration year round, there is only one year in which we could conduct this exercise. In the interest of time (our senior author has moved on to another career), we decided that the effort of interpolating between our sampling dates to estimate annual fluxes for just one year was probably not warranted by the possibility of better quantifying treatment effects. The only way that a cumulative value would be less noisy than our individual respiration rounds would be if the noise were negatively correlated across dates (this would be the case with cumulative litterfall mass). This doesn't seem likely for soil respiration measurements.

3. The hypotheses need to be written to help guide the reader through the findings, predictions, and the mechanisms. The hypothesis should also be revisited in the discussion, which lacks the broader contextualization of the findings. For example, the logic behind the suppression of soil respiration under nitrogen addition is related to plant nutrient limitation, but the actual results aren't explained in the discussion. If soil respiration isn't completely lower because of lower fine root biomass under nitrogen addition, is it because the fine root biomass measurements are too coarse, there are other effects such as differences in pH, etc.?

We added to the Introduction to provide context for all hypotheses tested (see revised or tracked-change manuscript). The objectives (final paragraph of the Introduction) was revised as follows: "In this study, we tested for effects of N and P addition on soil respiration across stands of three age classes in the MELNHE experiment. Our dataset spans 10 years of treatments (2011-2020) and includes three seasons (spring, summer, fall) of soil respiration measurements. Fine root biomass was measured in 2015-2016 to serve as a covariate for soil respiration. We predicted that soil respiration would be highest in ~~old~~ young stands ~~but~~ prior to canopy closure and that the response to nutrient additions would be most pronounced in young stands. We also expected ~~that~~ N addition to reduce soil respiration–as has been observed in similar experiments–and expected no effect from the addition of P. With regard to temporal effects, we expected transient responses to ~~the~~ treatments ~~could exhibit transient effects as~~ as various ecosystem components adjusted to changing soil nutrient availability. Finally, we predicted that the effect of N and P addition on soil respiration would vary by season due to seasonal dynamics of root growth and soil microbial communities (Abramoff & Finzi, 2016).

Lines 25-28: Abstract results are written in a slightly confusing way; successional stages need to be defined in the abstract before introducing the results of mid-successional

The second sentence of the abstract introduces our interest in succession: "The suppressive effect of nitrogen (N) addition on soil respiration is well documented, but the extent to which it may be moderated by stand age or the availability of soil phosphorus (P) is not well understood." When we introduce the results of the comparison with stand age, we define the mid-succesional age class: "Mid-successional stands (26-41 years old at the time of the first nutrient addition)…"

Line 33: what is this p-value here?

We added clarification: "Fine-root biomass was also lowest in mid-successional stands ($p = 0.01$ for the main effect of stand age) and had a positive effect on soil respiration of 1.33 µmol CO_2 m^{-2} s^{-1} g-1 (in summer 2012-2014, ($p = 0.10$)~~.~~ for the coefficient of fine-root biomass in linear regression). In addition to these important effects of treatment and stand age, we observed an unexpected increase in soil respiration, which doubled in ten years and was not explained by soil temperature patterns~~.~~, nutrient additions, or changes in instrumentation. "

Lines 34-35: are the increases in soil respiration across all treatments?

We added to this sentence of the Abstract: "In addition to these important effects of treatment and stand age, we observed an unexpected increase in soil respiration, which doubled in ten years and was not explained by soil temperature patterns, nutrient additions, or changes in instrumentation."

Here is the relevant section of the Results: "These time trends were independent of any treatment effects ($p \geq 0.37$ for all interactions of time, N, and P in linear regression), but temperature was positively correlated with soil respiration in each season ($p < 0.01$ for the coefficient of temperature in for spring and fall; $p = 0.08$ for summer)."

Lines 46-48: the mechanism between greater soil respiration and low soil fertility isn't super clear here: roots, rhizosphere, and mycorrhizae dynamics might need to be explained

This paragraph was revised: "Generally, rates of carbon partitioning belowground—and therefore soil respiration—are higher in low-fertility forests because greater effort is required for soil resource acquisition (Bae et al., 2015; Bloom et al., 1985; Gower et al., 1994; Litton et al., 2007). Relationships between N availability and forest carbon cycling are particularly well studied, and much of that research has demonstrated reduced soil respiration with increased N availability (Bowden et al., 2004; Burton et al., 2004; Bae et al., 2015; Kang et al., 2016). Mechanistically, the soil priming effect can explain the suppressive effect of N on soil respiration: when soil nutrients are scarce, autotrophic inputs of labile carbon and N stimulate[1] the turnover of more recalcitrant pools of carbon, releasing resources that were previously immobilized in that recalcitrant material and increasing belowground respiration from autotrophs and heterotrophs alike (Kuzyakov et al., 2000). In contrast, when nutrients are readily available–as is the case when N is added in experimental conditions–nutrient acquisition does not depend upon those immobilized resources that are released through soil priming, and soil respiration decreases. Further, under conditions of N excess, added N can reduce rates of decomposition through the down-regulation of the activity of ligninolytic enzymes (Carreiro et al., 2000; Knorr et al., 2005)."

Lines 57-59: why would you expect stand age to affect soil respiration in your study, and through what mechanisms? Are growth rates between the stand ages different aboveground to warrant expected differences in soil respiration? Are the other studies investigating stand age of similar stand ages to your study?

We added to first the paragraph of the Introduction to better support our hypotheses: "... rates of soil respiration change as forests progress through successional development. Because soil temperature is one of the primary drivers of soil respiration (Luo et al., 2001; Bronson et al., 2008; Bond-Lamberty & Thomson 2010), young stands exhibit high rates of soil respiration prior to canopy closure (Ewel et al., 1987; Xiao et al., 2014). Fine-root biomass, which reaches a maximum at canopy closure (Peichl & Arain 2006, Helmisaari et. al., 2002), likely contributes to the high soil respiration associated with young stands as well[1] . [2] Available nitrogen (N) decreased for the first 20 years following clearcutting and reached a maximum roughly 50 years later in aspen stands in Michigan (White et al., 2004). Perhaps as a result of these multiple factors, studies of soil respiration across stand age have come to inconsistent conclusions: some studies report increases in soil respiration with age (Gough et al., 2005), some report a decrease (Ewel et al., 2011; Tedeschi et al., 2006), and another reports interannual variation in the effect of stand age on soil respiration (Irvine & Law, 2002). On top of these developmental factors, seasonal changes in microbial communities (Sorensen et al., 2018) and rates of root growth (Abramoff & Finzi, 2016) could be expected to affect soil respiration as well."

Lines 79-82: these are nice hypotheses but the specific predictions need to be set up in the introduction (more the logic behind why one would expect these predictions). For example, do you expect the strongest nutrient effect in the youngest forests because they are the most limited by nitrogen for growth?

After improving the background material to better justify the hypotheses, we also improved the hypotheses to be consistent with the rationale, as documented under point 3, above.

Line 119: what does a more systematic location mean?

The previous sentence says, "In 2009, five collars were ~~haphazardly located~~ installed ~~located~~ in each plot, avoiding tree boles, boulders, large roots, and areas with severe drainage restriction." and the sentence in question says, "In 2010, these collars were moved to more systematic locations, and in 2014, two collars were added to each plot, for a total of seven collars per plot (Fahey et al. 2021)." We added a citation to the EDI package for the dataset used in this study, in which collar placement is described in detail.

Lines 124-126: how often were the measurements made and across which seasons? You could put some of this information into a supplementary table

The information is in an Appendix. We also added a reference to the published data package that includes every detail. "Measurements were made between 9 AM and 4 PM with most occurring between the hours of 10 AM and 2 PM (Fahey et al. 2023). The stands in which measurements were made and the number of times those stands were visited varied across years because of limitations of funding, personnel, or site access."

Line 138: how were the size classes of these roots defined, is there a citation for these specific size classes?

A cut-off of < 2 mm diameter is common (Tierney and Fahey 2007) but in this paper, we picked all roots < 5 mm from root cores (as manual coring is not suitable for measuring coarse roots). Since specific root respiration rate increases linearly with root N concentration (Burton et al. 2002) and root N concentration declines with increasing diameter (Fahey et al. 1988), the larger roots would contribute less to respiration per unit mass than the finer ones, and thus we focused on the finest root class. We improved our description of our approach: "Fine roots (~~less than 5~~ < 1 mm in diameter) were picked from the soil cores by hand ~~and sorted into two size classes: <1 mm in diameter and 1-5 mm in diameter~~. Roots 1-5 mm in diameter were also picked but are not reported here."

Lines 252-253: how does the addition of P reduce N availability, does it decrease N:P ratios or bring the N:P ratios closer to values in the control plots?

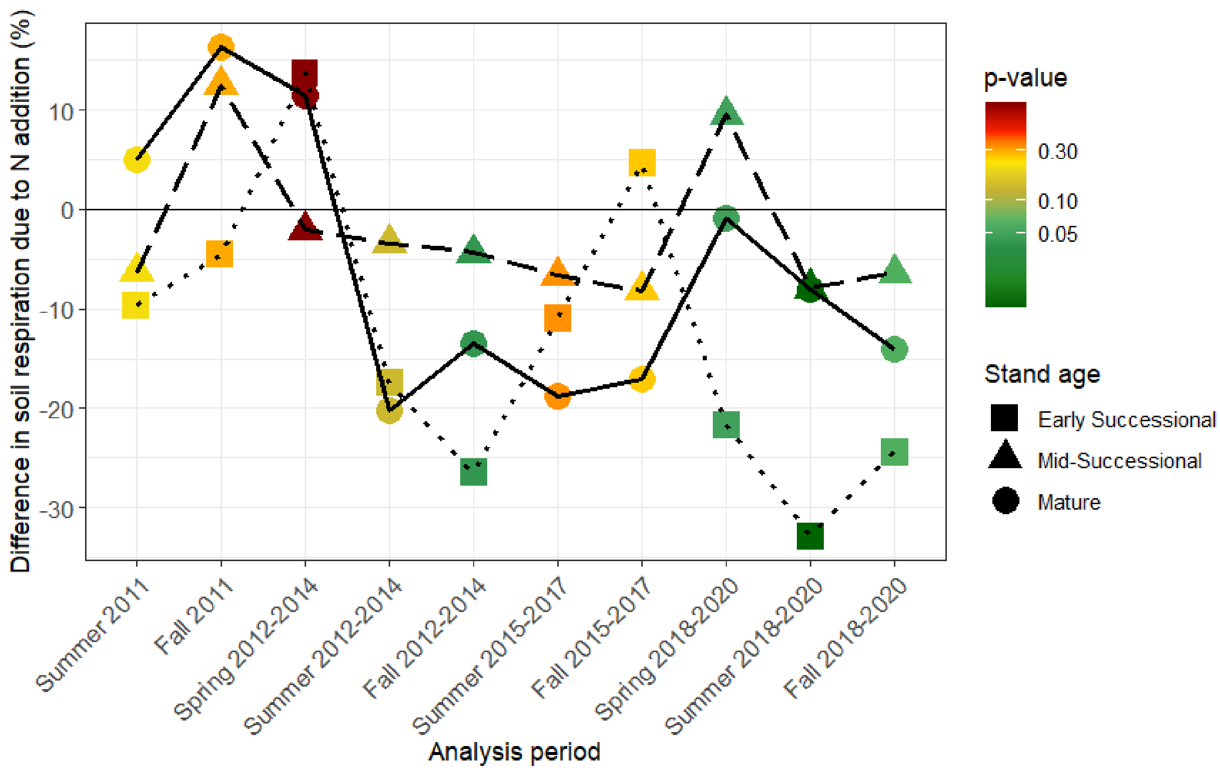
We clarified that this statement was about resin-available N. "The addition of P in the MELNHE study, however, has the effect of reducing resin-available soil N ~~availability~~ (Fisk et al. 2014)..."

Figure 3: It is difficult to connect the results to the figures: which results were significant or not based on the text? Indicate on Figure 3 which effects were significant like you did on Figure 1.

This figure is now Figure 4. We added to the caption: "P values for treatment effects can be found in Table 2." We tried adding them to the figure, but multiple P values are involved (main effect of N, main effect of P, interaction of these effects).

Figures 5-6: while the color by p value is nice in theory, it is a little hard to tell which time periods and seasons are significant. Maybe color the values differently by cutoff points, or change the colors so it's easier to see which values are significant. Otherwise, you could go by effect sizes, and when the effect size error crosses zero, it means not significant.

These figures have been improved:



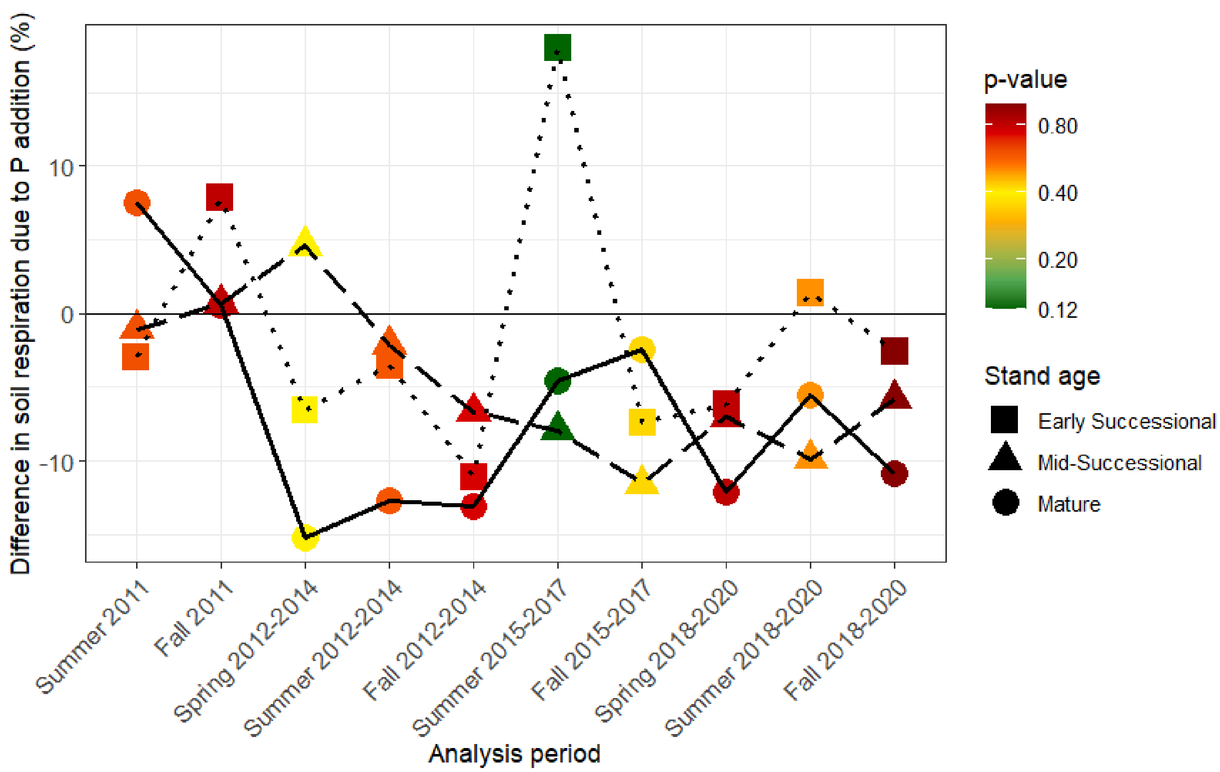


Figure 5: check Y axis label

Label changed to read "Difference in soil respiration due to P addition (%)". The corresponding change was also made to Figure 6.

Figure 6: what is "value"

This figure (now Figure 7) has been updated with more specific y-axis labels. The y axes were explained on the right-hand side of the figure as a default in R. Sorry about that.

References

Bowden, R.D., Davidson, E., Savage, K., Arabia, C. and Steudler, P., 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest ecology and management, 196(1), pp.43-56.

Taylor, L.L., Driscoll, C.T., Groffman, P.M., Rau, G.H., Blum, J.D. and Beerling, D.J., 2021. Increased carbon capture by a silicate-treated forested watershed affected by acid deposition. Biogeosciences, 18(1), pp.169-188.

We cited Bowden et al. (2004) but not Taylor et al. (2021).

Reviewer: 2

Comments to the Author(s)

Overall, this is a very well-written paper with some interesting data on interactive effects of long-term N and P additions on soil respiration. Such interactions are rarely studied, so this data is of value to the community. My main concerns are with the use of root biomass as a co-variate, when it was only collected at one time for each plot and some concerns regarding how overall main effects of N and P additions were assessed. These are described in more detail in the specific comments below. I appreciate the succinctness of the paper, but actually think a little more discussion may be warranted for a few of the items suggested below.

Specific Comments:

Line 29. I think there is an extra "soil" in this sentence.

Corrected, thanks. "This interaction effect is consistent with observations of reduced foliar N and available soil N ~~availability soils~~ following P addition."

Lines 34-35 and 287-296 . Any additional speculation about what might have caused this doubling of soil respiration over the decade? It's a bit unexpected, so I was hoping for maybe some more information on possible causes in the discussion. But, there may really be none. Do you have any data on soil moisture, for instance? Were the plots receiving more summer precipitation in later years, which could cause soil respiration to increase. I assume that methods and equipment remained consistent, ruling out changes in methods as a cause. The methods sections does state that the same instrument was used throughout, but perhaps reconfirming no changes in methods in the discussion of these results would clearly eliminate this possibility to readers.

We added just a few words to the Abstract: "In addition to these important effects of treatment and stand age, we observed an unexpected increase in soil respiration, which doubled in ten years and was not explained by soil temperature patterns, nutrient additions, or changes in instrumentation."

Similar changes were made to the Discussion: "The causes of the clear and significant 118% increase in summer soil respiration over the 10-year study are unknown (Figure 6). This temporal pattern was not explained by increases in soil temperature (Figure 6), ~~and it was independent of~~ treatment, or changes in instrumentation. A similar pattern has been observed for other locations in the Hubbard Brook Experimental Forest (Angela Possinger, personal communication)."

Two other studies of soil respiration at Hubbard Brook have observed this increase over time, and a future paper is planned to explore the cause.

Lines 101-106, Table 1. It would be nice to know more about the relative dominance of species in each stand. Would it be possible to add percent values for basal area in parentheses after each species listed in Table 1, or at least for the top two in each stand? Alternatively, an appendix with a summary for each stand (trees/ha or basal area/ha by species) would be helpful.

We added Figure 1, a stacked barplot that illustrates the basal area of the most common species across our stands:

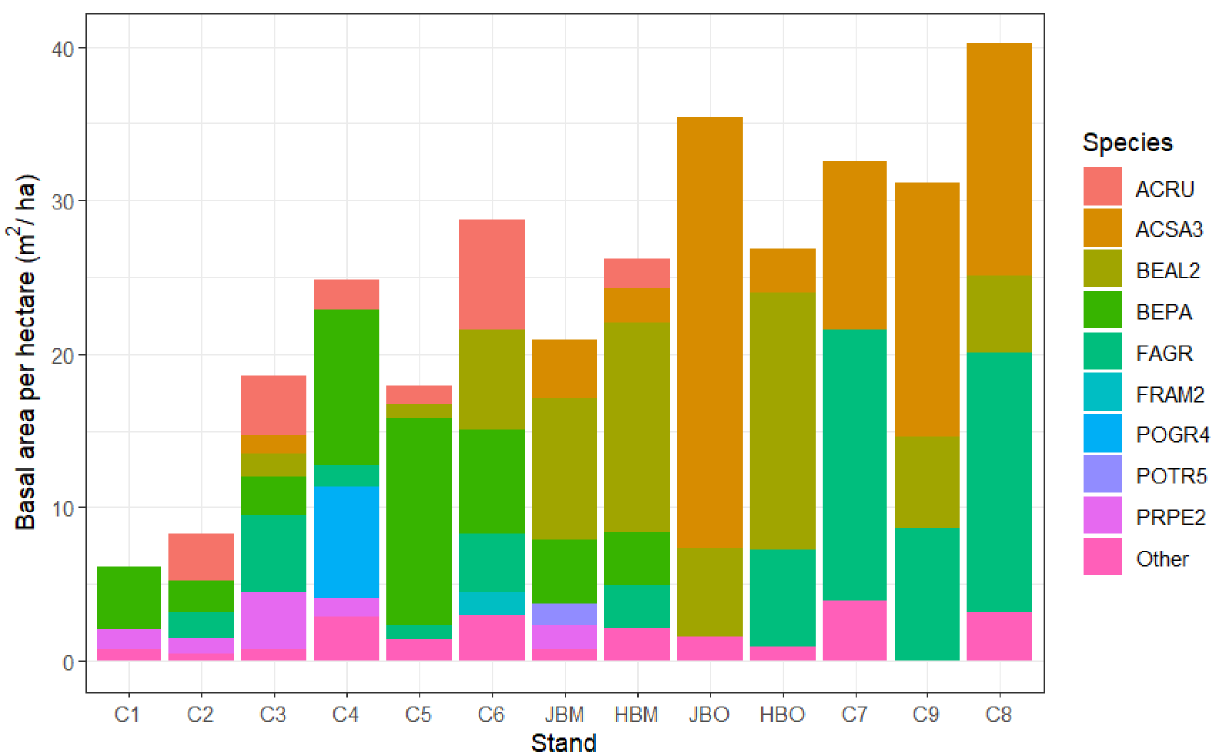


Figure 1. Basal area per hetare by species and stand. Stands are arranged from left to right in order of increasing stand age. Tree species are represented using USFS tree species codes.

Lines 116 to 120. Were the respiration collars left in place for the duration of the experiment or were they periodically reinstalled?

In addition to adding a citation to the EDI package for the dataset used in this study, we added a sentence to describe replacement of collars. Thanks for asking. "In 2010, these collars were moved to more systematic locations, and in 2014, two collars were added to each plot, for a total of seven collars per plot (Fahey et al. 2021). Collars were reinstalled and replaced as needed."

Line 128. Why 20 cm for soil temperature measurements? Much of the soil respiration is likely coming from roots and SOM decomposition located above 20 cm, where soil temperatures fluctuate more both seasonally and diurnally. I don't think this really affects your findings of soil respiration vs. temperature, but it might mute them somewhat.

This was a mistake, thank you for catching it. We corrected this sentence. "Soil temperature was measured simultaneously with soil respiration using a handheld resistance thermometer at a depth of ~~2~~10 cm adjacent to each collar."

Lines 158-160. Since root biomass can vary seasonally and annually, I'm not sure it's appropriate to use a single value for each plot as a covariate when assessing changes over years and variation among seasons within years for soil respiration. Possible effects this might have on interpretation of results should be discussed more in the paper. In fact, you suggest changes in root biomass could be part of the explanation for the increase in soil respiration over time, but don't really have data for looking at this.

Using a plot-average biomass is our best option, because the biomass cores (10 per plot) were co-located by our litterfall traps (5 per plot) and cannot be paired with our respiration collars (5-7 per plot, depending on the year). We have pre-treatment root biomass, not reported here, and we have almost completed picking roots collected in 2021-22 (it takes a few years to pick 40 cores from each of 13 stands). We chose the 2015-16 data to use as a covariate as being most representative of the time period under analysis. We did not detect systematic changes over time (variability is high). A paper on root biomass in the MELNHE study is currently in preparation by a graduate student, and we are neither prepared to provide that information in this paper, having one stand to finish picking from 2022, nor do we want to scoop that next paper.

Are differences among early, mid and late successional stands due to age or composition? It's not clear to me that the early and mids will have composition similar to the lates when they reach that age. Interpreting differences related to stand age is also complicated by the presence of only two early successional stands.

Yes, there are important differences in species composition with stand age, which are visible now in the new Figure 1. There are also important differences across sites in species composition (e.g., less beech at Jeffers Brook).

For example, on lines 187-189, is lower root biomass for mid-successional related to age or related to dominance of those stands by species which perhaps normally have lower root biomass.

Whether species composition explains some of the variation in fine root biomass is a great question, very well suited to a paper focusing on the root biomass as a response variable. We hope that such a paper will be forthcoming.

These are not necessarily problems, but instead just facts of the experiment that should probably be discussed in the discussion section (along with only single root biomass) as possible factors that may have influenced the data produced.

The authors analyze the data for each of ten periods. What results does an overall analysis across all ten periods yield relative to effects of N and P addition on soil respiration – or at least all periods after the first year? Would the main effect of P have been significant if the whole study period (all periods and seasons) was analyzed in a repeated measures way (lines 245-247). The data shown in Figure 5 suggests to me it may be but main effects are only provided for each of the ten periods.

We tried that, and P was not significant ($p=0.40$). Significant factors were: N ($p=0.03$), stand age ($p=0.07$), and soil temperature ($p<0.01$).

Figure 3. There is a "Pre-treatment" indication in the 2nd box of the top row, that I believe should not be there.

It's correct, but we see why it looks confusing. We added to the figure caption: "Treatments began in June 2011." This is now Figure 4.