Dear Associate Editor and Referees

On behalf of my co-authors, I would like to thank you for your thoughtful and constructive remarks, and for providing us with the opportunity to improve our manuscript. The text has now been thoroughly revised in order to meet the referees' concerns, with detailed responses to specific referee comments provided in the sections below. The Introduction and hypotheses have been re-written in order to draw stronger links between our prior research and the work presented here. We have also reformulated the hypotheses so that they more clearly test key ideas that emerged from our prior work. The Results have been simplified, particularly the sections pertaining to the laboratory manipulations, in order to aid reader understanding. Lastly, we have completely overhauled the Discussion, in order to better-integrate the discussion about the findings of our field and laboratory experiments. In addition, we have added a section discussing the implications of our measurements for the annual flux of N2O and N2, in-line with the recommendations of one of the referees. Please note that all line numbers referred to in this document are taken from the "clean" version of the text, where the "track changes" function in Microsoft Word has been disabled.

We hope that these changes will meet with your approval, and look forward to hearing from you in due course.

Yours sincerely,

Yit Arn Teh

RESPONSE TO REFEREE 1

1. In 2014, some of the authors of the present publication published in Biogeosciences (doi:10.5194/bg-11-2325-2014) a paper entitled "Methane and nitrous oxide fluxes across an elevation gradient in the tropical Peruvian Andes". It was a very interesting paper because there is only little information about soil nitrous oxide fluxes and their controls in tropical montane forest soils. In their one-year study they pointed out that nitrous oxide fluxes were primarily driven by denitrification and that nitrate availability was the principal constraint on soil nitrous oxide fluxes followed by soil moisture. In the present study Diem and colleagues extended their time-series to multi-annual time scales to identify controls of longer-term climatic variability, soil moisture and substrate availability on nitrous oxide fluxes in greater detail. They found out that habitat/elevation site, a proxy for nitrate availability under field conditions, was the best predictor for nitrous oxide fluxes. It is a great study. I have only few suggestions.

AUTHOR RESPONSE: We thank the referee for the positive remarks on our manuscript and constructive suggestions provided below.

2. I would suggest to reformulate the introduction and the hypotheses. The main message is that habitat/elevation – a proxy for NO3 availability in the field – is the best predictor for N2O flux and that seasonal differences of N2O flux and environmental variables were most pronounced at the lower montane forest site, where N2O flux was best explained by a combination of temperature, WFPS and N-availability. I would remove substrate availability and/or labile organic matter because it does not enrich the discussion but rather blur the main message. I think it is sufficient to discuss an absent correlation between N2O flux and variations in leaf-litter fall in one or two sentences and not in a whole discussion section (L827-L843).

AUTHOR RESPONSE: The referee makes a valuable observation about how the research is framed in the introduction, which is in-line with the suggestions of the second referee (point 17 below). The Introduction has now been heavily revised, in order to more clearly outline the key knowledge gaps identified by our earlier work, and to better establish the links between identified knowledge gaps and the research described in this manuscript (lines 99-153). The hypotheses have also been reformulated so that they link more explicitly to the unknowns and unresolved questions raised by our prior study, following the recommendations of the second referee (lines 134-142; also see point 17 below).

The only recommendation we have not fully acted upon is the suggestion the referee made with respect to the hypothesis on substrate limitation and labile organic matter (H3). With all due respect to the referee, we chose to retain this part of the hypothesis, because of the important role that labile organic matter is thought to play in modulating nitrate reduction (Morley and Baggs, 2010;Blackmer and Bremner, 1978;Davidson, 1991;Firestone et al., 1980;Weier et al., 1993). Moreover, the availability of labile organic matter is often used as a key input parameter for predicting N2O flux in several commonly used process-based models, such as DAYCENT, DNDC, and ECOSSE (Li, 2000;Smith et al., 2007;Werner et al., 2007). As a consequence, we believe that the negative finding from our field-based litter manipulation is still an important result to report on, because it suggests that labile organic matter may be a less important driver of N2O flux in these montane tropical ecosystems. However, we have acted on the referee's suggestion to condense the discussion of about labile organic matter so as not to belabour the point (lines 787-792).

3. At the moment it seems that results and discussion section are dominated by the description and interpretation of the experimental results in the lab. I am very sceptical whether the results from the laboratory-based nitrogen and WFPS manipulations can be directly linked to the results obtained in the field, especially when they are as puzzling and surprising as in the present study (i.e. WFPS-manipulation study). Substrate availability, nutrient limitations and a cascade of active microbial community composition may have drastically changed during transportation from the field site in Peru to Aberdeen. As long as there is no clearer picture about the active microbial community in the samples before and after transport, all of the nutrient and trace gas flux observations during incubation experiments have only potential implications. Additionally, the ratio of N2O to N2 production is pH-dependent. Did you check for potential pH changes upon transportation?

AUTHOR RESPONSE: We recognise that the results from the laboratory experiments represent only the potential behaviour of these soils. However, the laboratory experiments were an important aid to understanding patterns in the field data because it was difficult to establish clear empirical relationships between control variables and N2O flux, due to the confounding effects of multiple environmental controls. This point has now been clarified in the revised version of the text (lines 123-129). Furthermore, we have revised the Discussion so that the discussion of the field and laboratory results are better integrated, to provide a more holistic view of how environmental factors control N2O flux (please see the newly revised sections 6.1 and 6.2). By integrating the discussion of field and laboratory results, we hope that presentation of the findings does not appear so heavily dominated by our laboratory experiments.

The referee's point about potential treatment effects from handling, transportation, and storage of soils is well made. As far as possible, we tried to minimize potential treatment effects by transporting soils under ambient (room temperature) conditions, recognising that cold storage of tropical soils has been found to significantly alter soil process rates (Arnold et al., 2008;Verchot, 1999). We also set-up the laboratory experiments as quickly as possible after the soils were received in Aberdeen, normally within one or two weeks after the soils' arrival. Lastly, the laboratory incubations were conducted with intact soils, rather than sieved soils or slurries, recognizing that destruction of soil structure can alter biogeochemical process rates by changing redox gradients within aggregates and altering substrate competition among anaerobes (Sexstone et al., 1985;Teh and Silver, 2006).

With respect to the question of pH changes before and after transportation; we believe it is unlikely that transportation will have significantly altered pH, because average pH values did not appear to differ when we compared data from soils measured in Peru (Zimmermann et al., 2012;Zimmermann et al., 2009a;Zimmermann et al., 2009b) against samples that were measured after transportation to the UK. For the lab experiments described here, we did not measure pH measured after transportation, but only at the end of the incubations. The pH values measured at the end of the incubations were, on average, half a unit higher than the pH values measured for field soils.

4. What I find more fascinating is the observation of a negative relationship between WFPS and N2O flux in the field. The authors suggest that increasingly anaerobic conditions may stimulate N2O reductase activity and lead to greater denitrification to N2. This strengthens the assumption of Mueller et al. 2015 who suggested that gaseous N loss was likely dominated by N2 rather than N2O in Ecuadorian montane forest soils. Taken together, this finding may be generalized to tropical montane forest ecosystems.

AUTHOR RESPONSE: Thank you for the suggested reference; this paper and the insights gained from it have now been incorporated into the revised version of the text. The Discussion section was heavily revised to incorporate some of the more recent publications in this topic area, and

efforts have been made to stress the wider pan-Andean patterns which may be emerging from studies in both Peru and Ecuador (please see the newly revised sections 6.1 to 6.3).

5. This leads me to another suggestion. Many parts of the discussion section read like a repetition or better description of the results section (e.g. L740-L760; L814-L818; L851-L858; L869-L876; L881-L891). Moreover, the links between different parts are laborious (e.g. L730-L734; L751-L755; L784-790; L880). I think it is necessary to make the reading more "fluid". Many sentences in the results and discussion section begin with "For example" (e.g. L534, L620, L689, L745, L814). I think the discussion section would benefit if present results would be more interpreted in the light of recent publications (e.g. Baldos et al. 2015; Mueller et al. 2015; Nottingham et al. 2015).

AUTHOR RESPONSE: This point is well-taken, and is in-line with referee 2's suggestion that we should also streamline the results section (please see point 16 below). As noted in point 4 above, the Discussion has been completely overhauled in order to clarify some of the main messages, highlight commonalities between this study and parallel experiments elsewhere in the Andes, and in order to avoid undue repetition of information from the Results (please see the newly revised sections 6.1 to 6.3).

6. L45-L48: This should also be mentioned in the conclusion section

AUTHOR RESPONSE: Editorial suggestion taken (lines 895-897).

7. L98: ...derived from (missing word)

AUTHOR RESPONSE: The phrase "nitrate reduction" has now been added to the revised manuscript (line 105).

8. L290: What is the sampling size of the background concentration measurements?

AUTHOR RESPONSE: We measured background concentrations once for every individual soil core, thus n=5 for each elevation. The text has now been revised to incorporate this information (line 329).

9. L300: What was the length of time between sampling and analysis?

AUTHOR RESPONSE: Samples were analysed no more than one week after the samples arrived in Aberdeen. Transport time from Peru to the UK varied between one and two weeks. This information has now been added to the revised version of the text (lines 339-340).

10. L827-L843: Remove heading and shorten section.

AUTHOR RESPONSE: Editorial suggestion taken; also see point 2 above (lines 787-792).

11. L880-L900: Does this section really enrich the discussion?

AUTHOR RESPONSE: We believe so, because the aim of this paragraph was to link the patterns in the field data with what we found in the laboratory experiments. We also speculated as to why the nitrate reducing microbes in our soils showed such a weak response to relatively large manipulations of inorganic N availability, given that we expected that the microbes would show a stronger short-term response to elevated N inputs.

12. L906-L907: "Nitrous oxide flux originated primarily from nitrate reduction rather than from nitrification, probably due to low pH soil condition". Influence of pH has not been discussed in previous sections.

AUTHOR RESPONSE: The Discussion has now been revised to include a discussion of how pH may influence N2O production from ammonia-oxidation; namely, that under acidic conditions, recent advances in soil microbial research indicate that ammonia oxidation is primarily driven by ammonia-oxidizing archaea, which produces relatively little N2O compared to ammonia-oxidizing bacteria (AOB) (Hink et al., 2016;Prosser and Nicol, 2008). As a consequence, under the acidic soil conditions observed here, we believe suspect that most of the N2O is derived from nitrate reduction since N2O production from nitrification is so meagre (lines 731-758).

13. L912: It should be clearly stated whether results were obtained from incubation experiments or from the field.

AUTHOR RESPONSE: We have attempted to re-phrase the Conclusion so that it is clearer that these inferences are drawn from field observations (section 7).

14. Table1, Figure 3: Table and figure are very difficult to read. May be you can upload tables and figures in a higher resolution.

AUTHOR RESPONSE: Table 1 and Figure 3 are now presented as higher resolution images in the revised text.

15. References: Baldos et al. 2015 (DOI: 10.1890/14-0295.1) Mueller et al. 2015 (DOI: 10.3389/feart.2015.00066) Nottingham et al. (DOI:10.5194/bg-12-6071-2015)

AUTHOR RESPONSE: These references have been incorporated into the revised version of the text (see sections 6.1 to 6.3)

RESPONSE TO REFEREE 2

16. The authors address the complex issue of N2O emissions that is globally, even more for tropical forests, and particularly for montane tropical forests widely unconstrained. The

experimental setup in the field and in the laboratory were designed to capture mechanisms that affect N2O production and emissions. These effects include soil moisture, substrate availability (both mineral nitrogen and labile organic matter), soil moisture, oxygen, and temperature. They further analyzed more indirect predictors such as biome type, topography, seasonality, year to year variability as well as interacting effects among these potential drivers for N2O production. The major outcome of this study is that the controls on N2O emissions remain elusive and in parts counter existing knowledge. In particular, the study finds little seasonal variability despite strong seasonality in wetness. Further, soil moisture experiments suggest not the straightforward controls as they are being used in conceptual and numerical models. The exhaustive work done in soils in difficult and previously unsampled environment, as well as (in my view) important laboratory experiments that complement the field work. The data deserves dissemination to the scientific public. However, I do have some suggestions and comments on the presentation and interpretation of the data.

AUTHOR RESPONSE: We thank the referee for the positive remarks on our manuscript and constructive suggestions provided below.

17. Organization: The sheer number of observations and experiments, the exhaustive statistical analysis makes, and the resulting (complex pattern) makes it hard to write a clean story. Yet I think the authors should give the presentation some more thought. The result section is full of statistical test results, I am wondering if the tests applied and their results would not be better confined to tables, while the result text focuses more on the most important patterns.

AUTHOR RESPONSE: Thank you for these useful suggestions. It was, admittedly, difficult to find a very simple and elegant way of presenting the data, given the large number of observations, manipulative experiments, and complex results. The referee's suggestion, however, is well-taken, and is in agreement with the first referee's remarks about simplifying the text and clarifying the message (please see point 5 above). To address the referee's concerns, the Results section of the text has now been extensively revised and shortened, so that only the most important findings of our research are presented in the main body of the text. We have concentrated our efforts on revising the sections of the text that pertain to the laboratory incubations (sections 5.4 and 5.6), because these experiments show the most complex experimental design (i.e. three-way full factorial ANOVA). Statistical outputs for these laboratory experiment have now been summarised in two new tables for ease of reference (Supplementary Online Materials Tables S2, and S3). For the field data (sections 5.1 and 5.2), we have also made subtle alterations to the sentence structure, and judiciously removed unnecessary text. We have also produced new tables summarising the outputs from our statistical analyses in order to facilitate clarity of understanding (Supplementary Online Materials Tables S1).

18. Hypotheses: I would love to see a bit more nuanced hypotheses: Teh et al., 2014 already show an "odd" relationship with soil moisture (i.e. unexpected highs during dry season compared to wet season). Could better hypotheses be developed based on this earlier data? In

light of previous work done at the site, H1 and H2 are fairly generic. Similarly, since the paper also addresses elevation gradients (or transitions from premontane tropical forests to montane grasslands, perhaps there are potential to use that gradient to set up additional hypotheses (What are expectations if compared to [seasonally dry] lowland tropical systems?).

AUTHOR RESPONSE: Thank you for this remark. This comment is broadly in-line with observations made by referee 1 (please see point 2 above). As discussed previously, the introduction and hypotheses have now been heavily revised to draw stronger and more explicit links between our prior work and the findings of this study. The hypotheses themselves have been reformulated to better-reference the knowledge gaps and unknowns identified in our prior research.

19. Seasonality: Looking at the time series, it seems to me from the get go there is no direct seasonal effect. However, there are curious seasonal patterns: Soil moisture seems to lag quite a bit the precipitation (i.e. soil moisture seems to increase at the beginning of the dry season before it diminishes, while soil moisture continues to de- cline after the onset of the wet season). Much harder to discern, but just eyeballing the data in Fig 3, it seems there is a seasonal pattern of N2O emissions that it out of phase with seasonality, and is also out of phase with soil moisture. I do not have a mechanistic explanation how such lags can be formed given that often the first rain leads to strong pulses in denitrification. Nor do I know whether the patterns I seem to recognize are really there if further scrutinized. Yet I am wondering if there should be some exploration with the inclusion of lag in the analysis. Perhaps the authors toyed with it and did not pan out, However, I would be curious to know either way.

AUTHOR RESPONSE: We analysed the data in a number of different ways in order to explore not only instantaneous but lagged responses of N2O flux to rainfall. Unfortunately, because we did not have large enough number of data points, we were unable to employ more sophisticated time series approaches, such as autoregressive models, to evaluate whether the apparent lags in the data were real. We were therefore reliant on more simple methods of analysis, such as repeated measures ANOVA. We were unable to pinpoint lag effects using this method of analysis, although this is not to say these lags do not in fact exist; merely that we were unable to detect them using the sampling method and analysis tools that we employed.

20. Bimodal soil moisture response: The authors put strong emphasis on the bimodal soil moisture response of N2O emissions with peaks at 90 % and 50 % water filled pore space – stating it both in the abstract and the conclusion. However, this is in my view not clearcut, occurring only in some of the sampled soils. The results and the discussion acknowledge this. Is there a way to nuance the abstract and conclusion, such that the result do not come over as overstated?

AUTHOR RESPONSE: The manuscript has now been revised to clarify that this general bimodal trend is apparent only in the pooled dataset, subtly implying that there may be uncertainty as to whether this general trend is applicable for individual habitats (line 44).

21. Gradient nitrogen-rich -> nitrogen poor. In several places there is mention that the premontane and the lower montane habitats are nitrogen rich, whereas the higher elevations are considered nitrogen poor. It is perhaps worthwile to define N rich and N poor explicitly (for example by resin bag mineral N). This seems to be very important, given that nitrate availability may be a strong driver for N2O production.

AUTHOR RESPONSE: Thank you for this suggestion. The Discussion has now been revised to reference the resin-extractable nitrate data in order to better anchor the comparisons against a more objective empirical index (section 6.2).

22. Yet Figure 2 suggest that with respect to N2O emission, only the lowest forest has significantly higher emissions. But the authors also imply in some places (including in the abstract) that there is a continuous gradient in N2O emissions. Is this in conflict with each other (Although probably having altitude as predictor may lead to statistically significant N2O gradients)?

AUTHOR RESPONSE: We apologize for this error. The manuscript has now been revised to improve the precision of our language (e.g. lines 33-34).

23. Abstract L31: The statistical analysis does not show such a gradient, rather premontane forest was had much higher emissions than the rest (Figure 2). This may be a bit nit-picking on my part (I can see that the average in the lower montane forest is higher, but also has higher variability). Perhaps regress against altitude?)

AUTHOR RESPONSE: Editorial suggestion taken; please see point 22.

24. Abstract L40: Is the sentence starting with “This bimodal..” is a bit empty, not add much information. What is the complex relationship, what environmental variables?

AUTHOR RESPONSE: Editorial suggestion taken; the phrases “bimodal distribution” and “environmental variables” have been removed (lines 46-47).

25. Abstract L45: I think somewhere in the main text – perhaps discussion – it should be better laid out and evidenced that habitat is a proxy of NO3 availability.

AUTHOR RESPONSE: The case that habitat is a proxy for NO3- availability is now made in lines 794-815.

26. L 95: check spelling “areally”

AUTHOR RESPONSE: Editorial suggestion taken (line 102).

27. L 98: Sentence starting with “Nitrous oxide”: the use of parenthesis seems odd.

AUTHOR RESPONSE: Please see point 7.

28. L 104: Check the sentence – placement of “for” in the next line seems odd.

AUTHOR RESPONSE: The word “denitrification” had been accidentally omitted. The revised version of the text has now been re-written so this omission is no longer an issue.

29. L 152: I like how the authors also analyzed topographic landforms. However, through- out the paper it is not clear, how these landforms were binned and weighted to form a habitat-wide data sets. Also, where were the samples taken from for the laboratory manipulations? Further, can the terminology be kept a bit more consistent? Throughout the manuscript, it is referred to as topography, landscape feature, landform, and basin landform. I assume they are all the same, but I suggest to use a consistent designation for this categorical variable.

AUTHOR RESPONSE: Topography/landform was treated as a categorical variable in our repeated measures ANOVA or ANCOVA tests. For the laboratory incubations, two soil cores were sampled from each landform. With respect to terminology; we have attempted to revise the text so that a narrower range of terminology is now employed.

30. L250: This sentence essentially repeats the statement in L240

AUTHOR RESPONSE: This sentence has been removed in the revised manuscript.

31. L260: I assume the amount of litter added corresponds to the amount of litter falling in 1 month?

AUTHOR RESPONSE: Yes.

32. L483: Did you test for oxygen as a predictor, or was oxygen only assessed one time?

AUTHOR RESPONSE: Soil oxygen content was measured every time soil gas flux was sampled.

33. L506: >24 hour incubation: Over what period were the fluxes averaged?

AUTHOR RESPONSE: The overall period for the incubation was 48 hours. For the late phase of the incubation, we calculated the flux rate over 24 to 48 hours. The text has now been revised to make this clearer (lines 546-549, 655-658).

34. L667: Again, how long is the >24h period?

AUTHOR RESPONSE: Please see point 31.

35. L726: The figure shows that premontane habitat is significantly different from the other, and not that the lower elevation forests (premontane, and lower montane forest) are

significantly different from the higher elevation forests.

AUTHOR RESPONSE: The text has been corrected (see point 22).

36. L835: check the sentence starting with “Moreover,. . .”

AUTHOR RESPONSE: This section has been revised; see point 10.

37. L859: This sentence is not clear. What do the authors mean by “This pattern”

AUTHOR RESPONSE: We were referring to the overall trend of decreasing N2O flux with increasing elevation. The sentence has been removed in the revised version of the text.

38. L884: It is hard to believe that NO3 additions did not stimulate N2O emission. Just eye-balling Fig 5 suggests, it seems that N2O flux over the incubation period increased with increasing NO3 levels added. Is there some artifact because of the way the ANOVA has been done (admittedly this is a weak point on my part – but maybe a recheck and some explanation is possible to enlighten me and the readers)?

AUTHOR RESPONSE: When evaluating for the effect of N addition level on N2O flux, the ANOVA pooled data across all other categories (i.e. site, incubation phase) to compare the difference in N2O flux among N treatments. Because of the high level of variability in N2O flux among study sites and incubation phases, the net effect was that the ANOVA found no clear signal of N addition level alone. The lack of trend is not an artefact of the ANOVA calculation per se, but rather represents the high level of variability among soils from different study sites and differing responses of N2O flux during different incubation phases.

39. Supplementary figure: Please add the habitat to the x-axis for completion

AUTHOR RESPONSE: Editorial suggestion taken.

RESPONSE TO REFEREE 3

40. Diem et al. report on a remarkably large and comprehensive set of observations and experiments examining N2O fluxes across the Kosnipata tropical elevation gradient in Peru. This was clearly a lot of work. The combination of high temporal resolution chamber observations with WFPS, 15N and litter experiments makes the study particularly compelling. I have four suggestions. First, there a few aspects of the 15N tracer work that require further clarification. Second, I recommend the authors consider scaling their observations to annual values. Third, depending on details of the 15N tracer methods, I suggest the authors consider making use of the N2: N2O flux ratios from the incubations to estimate total N gas losses from these ecosystems if appropriate. Finally, I think the authors could do a better job at contextualizing

their work with reference to other studies and its global implications.

AUTHOR RESPONSE: We thank the referee for the positive remarks on our manuscript and constructive suggestions provided below.

41. 15N tracers: It would appear that the WFPS experiment was not a true "tracer" experiment but is also a N addition experiment and is therefore confounded. For the lower elevation sites, 200 ug N/g soil is not trivial. Are you sure that the background NO3 values are correct? The reported NO3-N values from soil extractions of ~150 ug/g are approximately 5-10 times higher than those observed in across most high N old- growth tropical forests worldwide. Tracer experiments often add < 0.5 ug/g at 15NO3 of ~99 atom percent. Further, unless I missed it, there is no description of the isotopic enrichment levels (per mil or atom percent). This needs to be included.

AUTHOR RESPONSE: Upon closer inspection, we realised that the values reported in the table are incorrect, and that the actual amount of N added was in fact much smaller than reported in the text. For example, for the WFPS experiment, the added amounts were 200 ng N/g soil for the lower elevation sites and 20 ng N/g soil for the higher elevation ones. For the N addition experiment, the values of N reported are the total amount of N added for the soil sample, and need to be normalised so that the values are reported on a per g soil basis. Thus, the true amounts of 15N tracer added in both the WFPS and N addition experiments are in fact in-line with the "trace" amount more typical of these types of 15N labelling experiments. This has been now corrected in the revised version of the text (Table 2). With respect to the level of isotopic enrichment; we applied the tracers at a 30 atom % level (see lines 272 and 350).

42. Scaling: Given the seasonal representation of the sampling, I think annual scaling could be justified. When scaled annually, the mean N2O-N emissions (0.27 mg N m-2 day-1) would be ~ 0.98 kg N ha-1 yr-1 with peak fluxes of ~2.7 kg N ha-1 yr-1. On average, chamber studies and models find that N2O losses from undisturbed humid tropical soils are ~1-4 kg N ha-1 yr-1 (See van Lent et al. Biogeosciences 2015 and Werner et al. Global Biogeochemical Cycles 2007). So, these values fit right in.

AUTHOR RESPONSE: In-line with referee' suggestions, we have now produced simple area-and seasonally-weighted annual flux estimates in the Discussion of the revised text (section 6.3, Table 4).

43. N2 fluxes: Given the response to the first point above, I suggest considering approximating total N gas losses from these ecosystems. Despite potential artifactual contributions of the incubations (disturbance, N additions) one could calculate rough N2 losses assuming equal N2:N2O ratios at a given WFPS as measured during the chamber work. This could be insightful as there are many chamber-based N2O estimates for tropical forests published but very few for total N gas fluxes because it's difficult to measure. Eyeballing the 15N2 versus 15N2O flux ratios (~20 to 80) and applying these to the chamber observations

would yield N2 fluxes of ~20 – 216 kg N ha-1 yr-1. The lower-end flux is possible (see Fang et al. PNAS 2015) but the upper end estimate is highly unlikely. Such total N export rates could never persist in a near-equilibrium forest as even the lower end is higher than average N mineralization and annual plant uptake and far exceeds external N inputs in tropical forests (see Brookshire et al. Geophysical Research Letters 2017).

AUTHOR RESPONSE: In-line with the referee's suggestions, we have now revised the Discussion to incorporate estimates of N2 flux and gaseous N export (section 6.3, Table 4).

44. The beauty of the Kosnipata gradient is that it represents a quasi-space-for-climate change substitution. More could be done with this context in the introduction and discussion. Further there are many other papers examining denitrification in tropical landscapes (some of them mentioned here) that would benefit the narrative to include.

AUTHOR RESPONSE: This remark is in agreement with concerns raised by other referees, and the Introduction and Discussion have been revised accordingly.

RESPONSE TO REFEREE 4

45. Diem et al. present a comprehensive set of lab and field data relating to controls of soil nitrous oxide flux across an elevation gradient in the Peruvian Andes. As both long-term field measurements and lab-based manipulations are included, they are able to approach the discussion of N2O fluxes in these ecosystems from several different directions. This was excellent work that will be a valuable addition to our current knowledge of N-oxide fluxes and tropical montane ecosystems. However, the authors could really improve the paper by taking some additional time to craft a more integrated presentation/summation of their study. The results section, in particular, should be revised. A well-designed table or figure (or combination) could provide a fascinating and useful summation of the different experiments, while eliminating the repetitive text. Instead, the text of the results section should highlight the most important results – much of this could be moved from the discussion section, which can then be condensed and re-focused to provide a bit more literature context about the different aspects of the results being discussed.

AUTHOR RESPONSE: We thank the referee for the positive remarks on our manuscript and constructive suggestions provided below.

46. Line 105: substrates for ____?

AUTHOR RESPONSE: This error has now been corrected in the revised version of the text. Please see point 24.

47. Line 138: give average temperature range over the course of the study

AUTHOR RESPONSE: Mean annual temperature is provided in Table 1.

48. Line 161: change ‘because of’ to ‘due to’

AUTHOR RESPONSE: Editorial suggestion taken.

49. Line 172: provide volume of chamber

AUTHOR RESPONSE: The chamber volume was approximately 0.008m^3 (8 L); the text has been revised accordingly (line 200).

50. Line 179: specify intervals

AUTHOR RESPONSE: Gas samples were collected at evenly spaced intervals over a 30 minute period; i.e. samples were collected 7.5 minutes apart. The text has been revised accordingly (line 206).

51. Line 187-192: were zeroes included?

AUTHOR RESPONSE: Yes. The text has been revised accordingly (lines 219-220).

52. Line 227-230: provide more detail: soil samples were taken in the field, air-dried and then re-wetted to target WFPS?

AUTHOR RESPONSE: To clarify, the WFPS experiments were conducted with field-moist samples; i.e. the soil samples were collected from the field, shipped to Aberdeen, and subsequently distributed into glass jars without being fully air-dried. For incubations where the target WFPS was below the field moisture levels, the soils were allowed to partially air-dry until they reached a value 10 % below the target WFPS for the experiment, and then carefully re-wetted through the 15N tracer application to bring up the soil moisture up to the target levels. For treatments where the target WFPS was above field moisture levels, the soils were simply wetted to 10 % below the target WFPS and then the 15N tracer solution added to bring the soil up to the target moisture level. The text has been revised accordingly (lines 258-262).

53. Line 231-233: needs clarification: 0-10 cm depth included the organic layer at all elevations, except in the upper montane forest where 0-10 cm depth included only mineral? If 0-10 sometimes included the organic layer, what was the thickness of the organic layer at those elevations? What was the thickness of the organic layer at the upper montane site; how deep did you go to access the 0-10 mineral sample? explain reasoning behind this sampling decision; could this have affected your results?

AUTHOR RESPONSE: For premontane forest, lower montane forest, and montane grassland, the organic matter in the upper 10 cm soil layer is intermixed with the mineral phase, and does not

constitute a distinct mineral-free horizon. Thus, we simply sampled from the 0-10 cm depth because there was no practical means of separating the organic matter from the mineral soil in these habitats. In contrast, upper montane forest soil shows a very different pattern of vertical stratification compared to the other habitats. In this habitat, the mineral soil is overlain by a thick (up to 17 cm deep) mineral-free organic layer, consisting of poorly decomposed leaves, roots, and humic materials; very akin to low density peat. To sample the mineral soil in this habitat, we went below this distinct organic horizon to a depth of approximately 17 cm. This information has now been added to the revised manuscript (lines 262-270).

With respect to the WFPS experiment; we decided to collect mineral soil from below the organic horizon in the upper montane forest because there was no mineral material found in this layer, making it difficult to compare results between habitats (given that the other habitats contain mineral material in the upper 10 cm of their soil profiles). At the time, we did not consider sampling the organic layer as well. This was an oversight on our part, which we tried to partially correct in our N addition experiments, by including the organic layer in those subsequent experiments.

54. Line 297-307: clearly distinguish between 'soil core' and 'soil sample'; "core" implies that the soil is still intact – once it has been mixed and added to the jars, the soil samples are no longer soil cores

AUTHOR RESPONSE: Editorial suggestion taken (lines 336-348).

55. Line 300-301: unclear; the five cores were mixed and then split into four equal parts? was the subsample and WFPS adjustment done on the cores or on the mixed soil in the jars?

AUTHOR RESPONSE: Each of the cores was split into four equal parts. The text has been revised to clarify this point (lines 341-342).

56. Line 375: change 'with' to 'and'

AUTHOR RESPONSE: Editorial suggestion taken.

57. Line 462: followed by topography

AUTHOR RESPONSE: Editorial suggestion taken.

58. Line 473: change 'is' to 'was'

AUTHOR RESPONSE: Editorial suggestion taken.

59. Line 474: define the fluctuation or refer to a table or figure where it is defined

AUTHOR RESPONSE: Editorial suggestion taken.

60. Line 585: change ‘for’ to ‘from’

AUTHOR RESPONSE: Editorial suggestion taken.

61. Line 761: change semicolon to comma

AUTHOR RESPONSE: Editorial suggestion taken.

62. Line 768: between soil temperature and ___?

AUTHOR RESPONSE: N2O; text has now been corrected.

63. Line 779: change ‘as’ to ‘at’

AUTHOR RESPONSE: Editorial suggestion taken.

64. Line 782: change ‘are’ to ‘is’

AUTHOR RESPONSE: Editorial suggestion taken.

65. Line 836: remove ‘and’

AUTHOR RESPONSE: Editorial suggestion taken.

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1. Title page:

Complex controls on nitrous oxide flux across a long elevation gradient in the tropical Peruvian Andes

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2. Abstract

Current bottom-up process models suggest that montane tropical ecosystems are weak atmospheric sources of N_2O, although recent empirical studies from the southern Peruvian Andes have challenged this idea. Here we report N_2O flux from combined field and laboratory experiments that investigated the process-based controls on N_2O flux from montane ecosystems across a long elevation gradient (600-3700 m a.s.l.) in the southern Peruvian Andes. Nitrous oxide flux and environmental variables were quantified in four major habitats (premontane forest, lower montane forest, upper montane forest and montane grassland) at monthly intervals over a 30-month period from January 2011 to June 2013. The role of soil moisture content in regulating N_2O flux was investigated through a manipulative, laboratory-based ^{15}N-tracer experiment. The role of substrate availability (labile organic matter, NO_3^-) in regulating N_2O flux was examined through a field-based litter-fall manipulation experiment and a laboratory-based $^{15}N\text{-}NO_3^-$ addition study, respectively. Ecosystems in this region were net atmospheric sources of N_2O, with an unweighted mean flux of 0.27 ± 0.07 mg $N\text{-}N_2O$ m^{-2} d^{-1}. Weighted extrapolations, which accounted for differences in land surface area among habitats and variations in flux between seasons, predicted a mean annual flux of 1.27 ± 0.33 kg $N_2O\text{-}N$ ha^{-1} $year^{-1}$. Nitrous oxide flux was greatest from premontane forest, which emitted 0.75 ± 0.18 mg $N\text{-}N_2O$ m^{-2} d^{-1}. In contrast, N_2O flux was significantly lower in other habitats, with lower montane forest emitting 0.46 ± 0.24 mg $N\text{-}N_2O$ m^{-2} d^{-1}, montane grasslands emitting 0.07 ± 0.08 mg $N\text{-}N_2O$ m^{-2} d^{-1}, and upper montane forest emitting 0.04 ± 0.07 mg $N\text{-}N_2O$ m^{-2} d^{-1}. Nitrous oxide flux showed weak seasonal variation across the region; only lower montane forest showed significantly higher N_2O flux during the dry season compared to wet season. Manipulation of soil moisture content in the laboratory indicated that N_2O flux was significantly influenced by changes in water-filled pore space (WFPS). The relationship between N_2O flux and WFPS was complex and non-linear, diverging from theoretical predictions of how WFPS relates to N_2O flux. Nitrification made a negligible contribution to N_2O flux, irrespective of soil moisture content, indicating that nitrate reduction was the dominant source of N_2O. Analysis of the pooled data indicated that N_2O flux was greatest at 90 and 50 % WFPS, and lowest at 70 and 30 % WFPS. This trend in N_2O flux suggests a complex relationship between WFPS and nitrate-reducing processes (i.e. denitrification, dissimilatory nitrate reduction to ammonium). Changes in labile organic matter inputs, through the manipulation of leaf litter-fall, did not

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alter N_2O flux. Comprehensive analysis of field and laboratory data demonstrated that variations in NO_3^- availability strongly constrained N_2O flux. Habitat – a proxy for NO_3^- availability under field conditions – was the best predictor for N_2O flux, with N-rich habitats (premontane forest, lower montane forest) showing significantly higher N_2O flux than N-poor habitats (upper montane forest, montane grassland). Nitrous oxide flux did not respond to short-term changes in NO_3^- concentration.

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3. Introduction

The tropics are the largest source of atmospheric nitrous oxide (N_2O), accounting for at least half of all global N_2O emissions (Hirsch et al., 2006;Huang et al., 2008;Kort et al., 2011;Nevison et al., 2007;Saikawa et al., 2014). The bulk of tropical N_2O emissions come from terrestrial sources, with the largest emissions arising from agricultural land and unmanaged lowland tropical forests (Hirsch et al., 2006;Huang et al., 2008;Kort et al., 2011;Nevison et al., 2007;Saikawa et al., 2014). However, while we have a relatively robust understanding of the global atmospheric budget as a whole (Hirsch et al., 2006;Huang et al., 2008;Saikawa et al., 2014), our knowledge of regional atmospheric budgets, particularly at the sub-continental scale, is much more limited, due to the constraints imposed by the spatial distribution of existing atmospheric sampling networks and ground-based, ecosystem-scale sampling efforts (Kort et al., 2011;Nevison et al., 2004;Nevison et al., 2007;Saikawa et al., 2014).

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In order to predict and model N_2O flux at these smaller (sub-continental) spatial scales, bottom-up emissions inventories or process-based models are often used, with emissions estimates constrained by empirical measurements (Werner et al., 2007;Li et al., 2000;Potter et al., 1996;Saikawa et al., 2013). However, these models are only as reliable as the data used to parameterize them; as a consequence, ecosystems that are under-represented in the empirical literature or which are poorly understood may be modelled less accurately, with knock-on effects for larger-scale emissions estimates (Saikawa et al., 2013;Teh et al., 2014;Werner et al., 2007). Nitrous oxide dynamics in montane tropical ecosystems are particularly poorly understood, because past research has concentrated on N_2O flux from lowland *tierra firme* forests (Saikawa et al., 2013;Teh et al., 2014;Werner et al., 2007).

Montane ecosystems, however, are important components of many tropical landscapes, and account for a sizeable land area. For example, in continental South America, montane ecosystems (>500 m a.s.l.) cover more than 8 % of the land surface (Eva et al., 2004), and play key roles in regional carbon (C), nitrogen (N), and greenhouse gas (GHG) dynamics (Girardin et al., 2010;Moser et al., 2011;Teh et al., 2014;Wolf et al., 2012;Wolf et al., 2011). Process-based models predict that N_2O flux from these montane environments are lower than those from the lowland tropics (i.e. <1.0 kg N_2O-N ha^{-1} yr^{-1}) (Saikawa et al., 2013;Werner et al., 2007). However, these models have rarely been tested against empirical data, and several field studies indicate that N_2O flux from montane ecosystems can exceed these prior models' estimates (Corre et al., 2010;Teh et al., 2014;Veldkamp et al., 2008). In some instances, N_2O flux from montane ecosystems can in fact approach emissions from lowland forests, begging the question as to whether or not existing models do, in fact, accurately represent flux from these high elevation ecosystems (Corre et al., 2010;Teh et al., 2014;Veldkamp et al., 2008).

In order to improve our wider understanding of the dynamics and biogeochemistry of N_2O in montane tropical forests, we conducted a combined field and laboratory study to investigate the environmental controls on denitrification and N_2O flux across a long elevation gradient (600-3700 m a.s.l.) in the tropical Peruvian Andes. Prior work from this region indicated that montane ecosystems in this area were stronger sources of N_2O than predicted by bottom-up process models (Teh et al., 2014). In particular, lower elevation premontane and lower montane forests, which account for the majority of the land area in this region (~54 %), showed emission rates that are on par with lowland tropical forests, suggesting that these ecosystems could be important contributors to regional atmospheric budgets (Teh et al., 2014). Nitrous oxide flux appeared to be derived from nitrate reduction (i.e. denitrification, dissimilatory nitrate reduction to ammonium), and was linked to seasonal variations in climate, with N_2O emissions increasing during the dry season compared to the wet season (Teh et al., 2014). However, contrary to theoretical expectations (Davidson, 1991;Firestone and Davidson, 1989;Groffman et al., 2009;Davidson and Verchot, 2000), N_2O flux was not directly correlated with soil moisture content in our field dataset (Teh et al., 2014), raising unresolved questions about the role of seasonal variations in soil moisture content in driving N_2O flux. We hypothesized that the weak relationship between N_2O flux and soil moisture

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content was because soil water-filled pore space (WFPS) – an index of soil moisture and a proxy for soil anaerobiosis – normally fell above the theoretical threshold where N_2O flux was constrained by the availability of anaerobic microsites (i.e. ~60 % WFPS) (Davidson, 1991;Firestone and Davidson, 1989;Groffman et al., 2009;Davidson and Verchot, 2000;Teh et al., 2014). Even during the dry season, WFPS rarely fell below this threshold value (Teh et al., 2014), allowing other driving variables, such as nitrate (NO_3^-), to play a more dominant role in regulating N_2O flux (Teh et al., 2014).

In the work presented here, we extended our time series to multi-annual time scales, in order to better understand the role of longer-term climatic variability in modulating N_2O flux. We also conducted a series of manipulative field and laboratory experiments to investigate the mechanistic controls on N_2O flux in greater detail, and to test hypotheses raised by our earlier work (as described below) (Teh et al., 2014). Furthermore, these manipulative experiments were crucial in helping us interpret our time series of field observations, because prior research indicated that the relationship between individual control variables (e.g. WFPS *or* NO_3^-) and N_2O flux were confounded by the simultaneous action of multiple control variables (Teh et al., 2014). The overarching goals of this research were to: investigate how climate and environmental variables regulate N_2O flux over multi-annual time scales; clarify the role of soil moisture as a proximate or distal control on N_2O flux; and evaluate the role of key substrates for nitrate reduction (i.e. labile organic matter, NO_3^-) in driving N_2O flux. Specifically, we hypothesized that:

- **H1.** Enhanced N_2O flux during the dry season (i.e. during periods of reduced soil moisture) is due to an increase in N_2O flux from nitrification and reduced N_2O reduction during denitrification
- **H2.** N_2O flux is poorly correlated with soil water-filled pore space *in situ* because soil moisture content does not normally constrain denitrification under field conditions; however, N_2O flux is closely correlated with water-filled pore space when soil moisture content is more limiting for denitrification (i.e. <60 % WFPS)
- **H3.** N_2O flux increases proportionately with the availability of substrates for denitrification (i.e. NO_3^-, labile organic matter)

In order to address these three objectives and their attendant hypotheses, we quantified N_2O flux and environmental variables from four major habitat types (premontane forest,

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lower montane forest, upper montane forest and montane grassland) at monthly intervals over a 30-month period. We also conducted manipulative laboratory experiments that investigated how variations in soil moisture content (WFPS) and NO_3^- availability influenced N_2O flux. In addition, we manipulated labile organic matter availability through a field-based litterfall manipulation study, recognizing that labile organic matter plays an important role in supplying not only the reducing equivalents for nitrate reduction, but also indirectly providing inorganic N for ammonia oxidation and nitrate reduction via N mineralization (Morley and Baggs, 2010;Blackmer and Bremner, 1978;Davidson, 1991;Firestone et al., 1980;Weier et al., 1993).

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4. Materials and methods

4.1 Study site

Measurements were conducted on the eastern slope of the Andes in the Kosñipata Valley, Manu National Park, Peru (Figure 1) (Malhi et al., 2010). This 3.02 x 10^6 ha (30,200 km^2) region has been the subject of intensive ecological, biogeochemical and climatological studies since 2003 by the Andes Biodiversity and Ecosystem Research Group (or, ABERG; http://www.andesconservation.org), and contains a series of long-term permanent plots across a 200-3700 m above sea level (m a.s.l) elevation gradient that stretches from the western Amazon to the Andes (Malhi et al., 2010). This part of the Andes experiences pronounced seasonality in rainfall but not in air temperature; the dry season extends from May to September and the wet season from October to April (Girardin et al., 2010). Thirteen sampling plots (approximately 20 x 20 m each) were established at four different habitats across a gradient spanning 600-3700 m a.s.l., including premontane forest (600 – 1200 m a.s.l.; n = 3 plots), lower montane forest (1200 – 2200 m a.s.l.; n = 3 plots), upper montane forest (2200 – 3200 m a.s.l.; n = 3 plots), and montane grasslands (3200 – 3700 m a.s.l.; n = 4 plots; colloquially referred to as “puna”) (Figure 1). In premontane forest, sampling plots were established in Hacienda Villa Carmen, a 3,065 ha biological reserve operated by the Amazon Conservation Association (ACA), containing a mixture of old-growth forest, secondary forest and agricultural plots (Teh et al., 2014). Sampling for soil gas flux was concentrated in the old-growth portions of the reserve. For lower montane and upper montane forests, sampling plots were established adjacent to or within existing 1 ha

permanent sampling plots established by ABERG (Teh et al., 2014). Sampling plots were also established in montane grasslands (Teh et al., 2014). To capture a representative range of environmental conditions, mesotope-scale (100 m-1 km scale landforms) topographic features were sampled (Belyea and Baird, 2006). Mesotopic features include ridges, slopes, flats and a high elevation basin. The latter two landforms include wet, grassy lawns with no discernible grade, and a peat-filled depression, respectively. Summary site descriptions are provided in Table 1. Data on soil properties were collected as part of this study, while mean annual precipitation is from earlier research by ABERG (Girardin et al., 2010).

4.2 Soil-atmosphere exchange

Field sampling was performed over a 30-month period from January 2011 to June 2013 for all habitats except for premontane forest. Due to circumstances outside our control, only 24-months of data were collected for premontane forest, with sampling commencing in July 2011. Soil-atmosphere flux was collected monthly, except where flooding or landslides prevented safe access by investigators to the study sites. Gas exchange rates were determined with five replicate gas flux chambers deployed in each of the thirteen plots (n = 65 flux observations per month). All representative landforms were sampled in each habitat (Table 1).

Soil-atmosphere flux of CH_4, N_2O and CO_2 were determined using a static flux chamber approach (Livingston and Hutchinson, 1995), although only N_2O flux is reported here. Methane and CO_2 flux are discussed in detail in another publication (Jones et al., 2016). Static flux chamber measurements were made by enclosing a 0.03 m^2 area with cylindrical, opaque (i.e. dark), two-component (i.e. base and lid) vented chambers with a ~8 L volume. Chamber bases were permanently installed to a depth of approximately 5 cm and inserted >1 month prior to the commencement of sampling, in order to minimize potential artefacts from root mortality following base emplacement (Varner et al., 2003). Chamber lids were fitted with small computer case fans to promote even mixing in the chamber headspace (Pumpanen et al., 2004). Headspace samples were collected from each flux chamber over a 30-minute enclosure period, with samples collected at 4 discrete intervals, 7.5 minutes apart, using a gastight syringe. Gas samples were stored in evacuated Exetainers® (Labco Ltd., Lampeter, UK), shipped to the UK by courier, and subsequently analysed for CH_4, N_2O

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and CO_2 concentrations with a Thermo TRACE GC Ultra (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) at the University of St Andrews. Chromatographic separation was achieved using a Porapak-Q column, and analyte concentrations quantified using a flame ionization detector (FID) for CH_4, electron capture detector (ECD) for N_2O, and methanizer-FID for CO_2. Instrumental precision was determined by repeated analysis of standards and was better than 5 % for all detectors. Gas flux rates were determined using the R HMR package to plot best-fit lines to the data for headspace concentration against time for individual flux chambers (Pedersen et al., 2010;Team, 2012). Gas mixing ratios (ppm) were converted to areal flux by using the Ideal Gas Law to solve for the quantity of gas in the headspace (on a mole or mass basis), normalized by the surface area of each static flux chamber (Livingston and Hutchinson, 1995). Measurements resulting in zero net flux were included in our dataset.

4.3 Environmental variables

To investigate the effects of environmental variables on trace gas dynamics, we determined soil moisture, soil oxygen content in the 0-10 cm depth, soil temperature, and air temperature at the time of flux sampling. Volumetric soil moisture content was determined using portable soil moisture probes (ML2x ThetaProbe, Delta-T Device Ltd., Cambridge, UK) inserted into the substrate immediately adjacent to each flux chamber (<5 cm from each chamber base; depth of 0-10 cm). Soil moisture content is reported here as water-filled pore space (WFPS), and is calculated using the measurements of volumetric water content and bulk density (Breuer et al., 2000). Soil O_2 concentration was determined using the approach described by Teh et al. (2014). Soil temperature (0-10 cm depth), chamber temperature and air temperature was determined using type K thermocouples (Omega Engineering Ltd., Manchester, UK). Data on aboveground litter-fall, meteorological variables (i.e. photosynthetically active radiation, air temperature, relative humidity, rainfall, wind speed, wind direction), continuous plot-level soil moisture (10 and 30 cm depths) and soil temperature (0, 10, 20 and 30 cm depths) measurements were also collected, but are not reported in this publication.

Resin-extractable inorganic N flux (i.e. ammonium, NH_4^+; nitrate, NO_3^-) were quantified in all plots using a resin bag approach (Templer et al., 2005;Subler et al., 1995). From August 2011

onwards, ion exchange resin bags (n = 15 resin bags per elevation) were deployed at the bottom of the plant rooting zone (i.e. 0-10 cm depth in premontane forest, lower montane forest and montane grasslands; 0-15 cm in upper montane forest), following established protocols (Templer et al., 2005;Subler et al., 1995). Samples were collected at monthly intervals (where possible) for determination of monthly, time-averaged NH_4^+ and NO_3^- flux (Subler et al., 1995). For some plots, this sampling frequency was periodically disrupted due to natural hazards (i.e. landslides, river flooding) preventing safe access to the study sites. Resin bags were shipped to the University of Aberdeen after collection from the field, inorganic N was extracted using 2 M KCl and concentrations determined colourimetrically using a Burkard SFA2 continuous-flow analyser (Burkard Scientific Ltd., Uxbridge, UK) (Templer et al., 2005;Subler et al., 1995).

4.4 Water-filled pore space manipulation study

We investigated the effects of WFPS on N_2O flux derived from nitrate reduction or nitrification rates using a ^{15}N tracer experiment. Soil cores for all habitats were collected from the 0-10 cm depth, and were not fully air-dried nor sieved prior to incubation. Soils were distributed into glass jars and adjusted to 10% below the target WFPS values of 30%, 50%, 70% and 90%, either by letting the soils partially air-dry or by adding water to them, depending on the WFPS of the soils at the time of collection (n = 5 for each ^{15}N addition and 3 controls for each WFPS for a total of n = 212; see Table 2). Additional de-ionized water, containing the ^{15}N tracers, was subsequently added gravimetrically to raise WFPS to target levels. The exception to this was for the upper montane forest, where samples were collected from the 0-10 cm depth of the mineral soil, but not from the organic layer. The reason for this is that the mineral soil layer in the upper montane forest is overlain by a thick organic horizon up to 17 cm deep, consisting of poorly decomposed leaves, roots, and humic materials; very akin to low density peat (Zimmermann et al., 2012;Zimmermann et al., 2009a;Zimmermann et al., 2009b). In contrast, the organic matter in the upper 10 cm soil layer in the other habitats is closely intermixed with the mineral phase, and does not normally constitute a distinct mineral-free horizon. Thus, to sample mineral soil in the upper montane forest, we had to sample beneath this thick organic horizon.

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Two different types of ^{15}N-tracers (30 atom %) were applied to the soils in order to determine the proportion of N_2O derived from nitrate reduction and nitrification (Bateman and Baggs, 2005). $^{14}N\text{-}NH_4{}^{15}N\text{-}NO_3$ was used to quantify the amount of N_2O produced by nitrate reduction, while $^{15}N\text{-}NH_4{}^{15}N\text{-}NO_3$ was used to quantify the amount of N_2O produced from both nitrate reduction and nitrification. The difference between the two was used to calculate the amount of N_2O derived from nitrification alone. After application of the tracers, the jars were sealed, and gas samples taken at 0, 6, 12, 24, 36 and 48 hours to determine rates of gas flux. Nitrous oxide yield was calculated as the ratio of $^{15}N\text{-}N_2O$ flux : $^{15}N\text{-}N_2O$ flux + $^{15}N\text{-}N_2$ flux. Soils were sampled at the end of the experiment for NO_3^- concentration, NH_4^+concentraion, and total C and N content.

Soil gas concentrations (N_2O, CO_2 and CH_4) were measured on a GC as described in section 4.2, while $^{15}N\text{-}N_2$ and $^{15}N\text{-}N_2O$ were measured on a SerCon 20:20 isotope ratio mass spectrometer equipped with an ANCA TGII pre-concentration module (SerCon Ltd., UK). The coefficient of variation (CV; an index of instrumental precision) for repeated analysis of gas concentration and isotope standards was <5 %. $^{15}N\text{-}N_2O$ and $^{15}N\text{-}N_2$ fluxes were calculated from the ^{15}N atom percent excess of the samples compared to the controls using the HMR package (Pedersen et al., 2010).

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4.5 Litter-fall manipulation experiments

We conducted a field-based litter-fall manipulation experiment to test for the effects of variations in labile organic matter availability on trace gas flux. This study took place over a 14-month period (April 2012 to June 2013), and consisted of 4 experimental treatments (control, +50 % litter addition, +100 % litter addition, litter removal) implemented across 3 habitats (premontane forest, lower montane forest, upper montane forest), with 6 replicate plots per treatment per habitat (each treatment plot was 0.5 x 0.5 m in size; n = 24 observations per habitat; n = 72 observations per sampling increment). Leaf litter addition rates for the +50 % and +100 % litter addition treatments were determined based on prior research from this study site, and fell within the natural range of variability observed across this elevational gradient (Girardin et al., 2010).

Litter-fall for the litter addition treatments was collected monthly in litter baskets (n = 3 litter baskets per treatment plot for a total of n = 18 per habitat). These data were also used to determine the background rates of leaf litter-fall among habitats. For the control, litter inputs simply reflected natural background litter-fall rates. For the +50 % and +100 % litter addition treatments, background litter inputs were supplemented with additional litter taken from the litter baskets. Briefly, wet litter was weighed in the field using portable scale, gently mixed (homogenized), and then re-distributed to the +50 % and +100 % litter addition plots in amounts proportional to the average amount of wet litter that fell into the litter baskets over the course of the month. As a consequence, the amount of litter added in the two litter addition treatments was not fixed but varied according to the natural background rate of litter-fall. For the litter removal treatment, leaf litter was removed from the forest floor at the start of the experiment, and 3mm nylon mesh was placed over the surface of the treatment plot to prevent further litter ingress to the soil surface. Any debris accumulating on the mesh was removed at monthly intervals.

Trace gas flux and environmental variables were determined at 7 time points over the course of the 14-month experiment using the methods described in section 4.2. In addition, soil moisture (WFPS from the 0-10 cm depth), soil temperature (0-10 cm depth), air temperature, soil gas concentrations (O_2, CH_4, N_2O, CO_2) from the 0-10 cm and 20-30 cm depths, litter C, and litter N were determined concomitantly. Litter C and N content was determined on a Carlo-Erba NA 2500 elemental analyser (CE Instruments Ltd, Wigan, UK) at the University of Aberdeen.

4.6 Nitrate addition experiment

To quantify the effect of NO_3^- availability on N_2O flux, we conducted a $^{15}N\text{-}NO_3^-$ addition experiment. Background concentrations of NO_3^- were determined prior to the start of experiment using soil subsamples (n = 5 per elevation), after which the soils from each habitat were divided into three treatment groups, and supplemented with surplus NO_3^- which raised these background levels by +50 %, +100 %, and +150 % (Table 2). The NO_3^- added to the soil in each of the treatments was enriched with ^{15}N in order to trace the conversion of nitrate to gaseous N products ($^{15}N\text{-}N_2O$, $^{15}N\text{-}N_2$) (Baggs, 2003;Bateman and Baggs, 2005).

Soil cores were sampled from 0-10 cm for each habitat (n = 6 soil cores per habitat), with the exception for upper montane forest, where two separate sets of cores were collected, one from the organic layer (O horizon; n = 6) and the other from the mineral layer (A horizon; n = 6). Soil samples were then shipped to the University of Aberdeen and sampled within one week of arrival. Transport times from Peru to the UK varied between one and two weeks. Five of these soil cores, one for each replicate, were split into four equal parts (3 treatment samples and one control sample) and distributed into 1 L screw top jars (Kilner, UK). A small soil subsample from each core was used to determine WFPS, background NO_3^- content (extracted in 100ml 1M KCl for a 10g soil sample prior to the start of the experiment), as well as total C and N content. If necessary, the samples were gravimetrically amended with water until the cores reached 80% WFPS. Soil cores were kept under constant conditions for 3 days before the start of the experiment to minimize the effects of changing water content on soil processes.

At the start of the experiment, dissolved ^{15}N-labelled KNO_3 (30 atom %) was added according to the measured NO_3^- concentrations of each core to reach the required NO_3^- concentration for each treatment (Table 2). Initial NO_3^- concentration (prior to ^{15}N addition) averaged (± standard error) 157 ± 12 µg N g $soil^{-1}$ for pre-montane forest, 140 ± 12 µg N g $soil^{-1}$ for lower montane forest, 19 ± 7 µg N g $soil^{-1}$ for upper montane forest organic layer soil, 18 ± 5 µg N g $soil^{-1}$ for upper montane forest mineral layer soil, and 6 ± 2 µg N g $soil^{-1}$ for montane grassland soil (Table 2). The jars were then sealed with lids fitted with a two-way stopcock to allow for gas sampling. Gas samples were taken with gas tight syringes, and stored in pre-evacuated containers for determination of ^{15}N-N_2, ^{15}N-N_2O, N_2O, CO_2 and CH_4 content. Isotope samples (150 ml) were stored in 100 mL serum bottles and gas concentration samples (20 ml) were stored in 12 ml Exetainers® (Labco Ltd., Lampeter, UK). After gas sampling, the stopcock was opened to allow the sampled air from the jar to be replaced by lab air, and lab air was sampled to allow for correction of the gas concentrations in the jars due to dilution. Samples were taken at 0, 6, 12, 24, 36, and 48 hours, after which the jars were opened and soil was sampled for determination of NO_3^-, NH_4^+ and total C and N. Gas flux, isotopic and elemental concentrations were determined according to the methods described previously.

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4.7 Statistics

Statistical analyses were performed using JMP IN Version 8 (SAS Institute, Inc., Cary, North Carolina, USA) or R (Team, 2012). Residuals were checked for heteroscedasticity and homogeneity of variances. Where necessary, the data were transformed using a Box-Cox procedure to meet the assumptions of analysis of variance. Analysis of variance (ANOVA) or Generalized Linear Models were used to evaluate the effect of categorical variables (i.e. site, season, topography) on trace gas flux and environmental variables. Analysis of covariance (ANCOVA) was performed on Box-Cox transformed data to investigate the combined effects of categorical variables and environmental factors (e.g. water-filled pore space, soil oxygen content, air temperature, soil temperature, etc.) on trace gas flux. Non-parametric tests were employed where Box-Cox transformation was unable to normalize the data, homogenize the variances, or where the residuals still showed strong trends even after Box-Cox transformation. Means comparisons were performed using Fisher's Least Significant Difference test (Fisher's LSD). Statistical significance was determined at the $P < 0.05$ level, unless otherwise noted. Values are reported as means and standard errors (± 1 SE). Statistical analyses for the field data were conducted on plot-averaged data to avoid pseudo-replication.

5. Results

5.1 Variations in N_2O flux among habitats and between seasons

The overall mean N_2O flux for the entire dataset was 0.27 ± 0.07 mg N-N_2O m^{-2} d^{-1}, with a range from -8.40 to 75.0 mg N-N_2O m^{-2} d^{-1}. We investigated the effect of habitat, season, topography, and the interaction of habitat by season on N_2O flux by using a three-way ANOVA on plot-averaged data ($F_{10,307}$ = 3.28, $P < 0.0005$; Supplementary Online Materials Table S1A). We found that there was a significant effect of habitat ($P < 0.003$) and an effect of season at the borderline of statistical significance ($P < 0.07$). However, we found no effect of topography and no habitat by season interaction effect on N_2O flux. Habitat accounted for the largest proportion of variance in the dataset (4.3 %), while season accounted for only 1.0 % of the variance (Supplementary Online Materials Table S1A).

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Among habitats, the overall trend was towards the highest flux from premontane forest (0.75 ± 0.18 mg N-N_2O m^{-2} d^{-1}), followed by lower montane forest (0.46 ± 0.24 mg N-N_2O m^{-2} d^{-1}), montane grasslands (0.07 ± 0.08 mg N-N_2O m^{-2} d^{-1}), and upper montane forest (0.04 ± 0.07 mg N-N_2O m^{-2} d^{-1}) (Figure 2a). Multiple comparisons tests indicated that only premontane forests showed statistically higher flux than the others (Fisher's LSD, $P < 0.05$); while there were numerical differences in mean flux among the other habitats, large variances meant that they had overlapping ranges of flux (Figure 2a).

The borderline significant effect of season ($P < 0.07$) reflected an overall trend of higher dry season (0.51 ± 0.18 mg N-N_2O m^{-2} d^{-1}) compared to wet season flux (0.15 ± 0.07 mg N-N_2O m^{-2} d^{-1}) in the pooled dataset (Table 3). However, part of why the effect of season was weak was because only lower montane forest showed significant variability between seasons (Fisher's LSD, $P < 0.05$), while the other three habitats did not show significant seasonal differences in flux (Fisher's LSD, $P < 0.05$).

Even though the effect of topography alone was not statistically significant, N_2O flux from flat sites were significantly higher (0.62 ± 0.28 mg N-N_2O m^{-2} d^{-1}) than from the basin site (-0.18 ± 0.16 mg N-N_2O m^{-2} d^{-1}) (Fisher's LSD, $P < 0.05$). However, there was no significant difference between flat sites and either slope or ridge sites (0.24 ± 0.09 mg N-N_2O m^{-2} d^{-1} and 0.20 ± 0.08 mg N-N_2O m^{-2} d^{-1}, respectively) (Fisher's LSD, $P > 0.05$).

For each habitat, we also compared individual wet and dry seasons against each other using multiple comparisons tests (e.g. dry season 2012 vs wet season 2012; dry season 2012 vs dry season 2013, etc.) to determine if there was significant inter-annual (i.e. year-on-year) variation in N_2O flux among seasons. Consistent with our three-way ANOVA results, we found that only lower montane forest showed significant variation among multiple dry and wet seasons, whereas the other habitats showed no significant trends. For lower montane forest, we observed significantly higher dry season flux in 2011 compared to wet and dry seasons in all other years ($P < 0.05$; Figure 3b).

5.2 Variations in environmental conditions among habitats and between seasons

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We investigated the effect of habitat, season, topography, and the interaction of habitat by season on environmental variables using a three-way ANOVA on plot-averaged data. The environmental variables examined here were: water-filled pore space (WFPS) in the 0-10 cm depth, gas-phase soil oxygen content in the 0-10 cm depth, soil temperature, air temperature, and resin-extractable inorganic N flux (NH_4^+, NO_3^-).

Water-filled pore space varied significantly as a function of habitat, season, habitat by season, and topography ($F_{10,304}$ = 637.96, P < 0.0001; Table 3; Figure 2b; Figure 3; Supplementary Online Materials Table S1B). Habitat accounted for the largest proportion of variance in the model (78.1 %), followed by season (0.6 %), habitat by season interaction (0.6 %), and topography (0.4 %) (Supplementary Online Materials Table S1B). Each habitat differed significantly from the others (Fisher's LSD, P <0.05), with the highest WFPS observed in montane grassland (88.4 ± 0.3 %), followed by premontane forest (51.6 ± 1.3 %), lower montane forest (39.0 ± 0.9 %), and upper montane forest (35.0 ± 1.5 %) (Figure 2b). WFPS varied significantly between seasons (t-Test, P < 0.05), with a mean dry season value of 52.1 ± 2.4 % compared to a mean wet season value of 59.5 ± 1.6 % (Table 3). The significant habitat by season interaction is due to the fact that some habitats showed seasonal trends in WFPS whereas others did not. Whereas lower montane and upper montane forests all showed a significant reduction in WFPS during the dry season, premontane forest and montane grasslands showed no seasonal differences in WFPS (Table 3, Figure 3). For topography, the main effect was that the basin landform had significantly higher WFPS than the other landforms. The basin landform showed a mean WFPS of 89.3 ± 0.1 % whereas WFPS in other landforms ranged from 51.7 ± 2.2 to 57.7 ± 2.7 %.

Soil oxygen in the 0-10 cm depth varied significantly as a function of habitat, habitat by season, and topography ($F_{10,242}$ = 27.70, P < 0.0001; Table 3; Supplementary Online Materials Table S1C). Habitat accounted for the largest proportion of variance in the model (66.9 % of the total variance), followed by topography (8.4 %), habitat by season (3.5 %) (Supplementary Online Materials Table S1C). For habitat, multiple comparisons tests indicated that only montane grasslands showed significantly lower soil O_2 content than the other habitats (13.5 ± 0.6 %), while the others showed statistically similar soil O_2 values to each other (18.6 ± 0.2 to 19.5 ± 0.1 %; Fisher's LSD, P < 0.05). For topography, multiple

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comparisons tests indicated that the basin landform showed statistically lower soil O_2 content than the other landforms (7.4 ± 2.3 %), whereas the other topographic features showed statistically similar values, ranging from 16.9 ± 0.6 to 18.2 ± 0.2 % (Fisher's LSD, P < 0.05). The significant habitat by season interaction was due to the fact that only montane grassland showed a significant difference in O_2 content between wet and dry season, whereas other habitats showed similar soil O_2 values (Table 3).

For soil temperature, the effects of habitat, season, habitat by season, and topography were all significant ($F_{10,292}$ = 790.7, P < 0.0001; Supplementary Online Materials Table S1D). Habitat accounted for the largest proportion of variance in the model (85.5 % of the total variance), followed by season (1.4%), habitat by season interaction (0.5 %), and topography (0.3 %) (Supplementary Online Materials Table S1D). Each habitat differed significantly from the others (Fisher's LSD, P <0.05), with the highest soil temperature observed for premontane forest (20.5 ± 0.1 °C), followed by lower montane forest (17.8 ± 0.1 °C), upper montane forest (11.5 ± 0.1 °C), and montane grasslands (10.6 ± 0.2 °C). Soil temperature varied significantly between season (t-Test, P < 0.05), with a mean dry season value of 13.9 ± 0.4 °C compared to a mean wet season value of 15.1 ± 0.3 °C. The significant habitat by season interaction is due to the fact that some habitats showed more pronounced seasonal trends in soil temperature than others, although the overall pattern of cooler dry season compared to wet season soil temperatures holds across all habitats (Table 3). For topography, the flat landforms showed significantly higher soil temperatures than the others (16.0 ± 0.5 °C), the basin landform showed significantly lower values (10.8 ± 0.4 °C), whereas ridge and slope landforms showed similar values to each other (14.3 ± 0.4 °C and 14.7 ± 0.4 °C, respectively) (Fisher's LSD, P < 0.05).

For air temperature, only the effect of habitat was significant ($F_{10,292}$ = 103.2, P < 0.0001; Table 3; Supplementary Online Materials Table S1E). A multiple comparisons test indicated that each habitat showed significantly different temperatures compared to the others (Fisher's LSD, P < 0.05). Premontane forest showed the highest air temperatures (21.0 ± 0.3 °C), followed by lower montane forest (18.7 ± 0.2 °C), upper montane forest (12.7 ± 0.2 °C), and montane grassland (11.7 ± 0.3 °C). Other variables did not significantly affect air temperature.

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For resin-extractable NH_4^+ flux, even though the three-way ANOVA model was not statistically significant, the overall trend was towards significantly lower NH_4^+ flux in the dry season (9.6 ± 0.7 µg N-NH_4 g $resin^{-1}$ d^{-1}) compared to the wet season (22.3 ± 3.6 µg N-NH_4 g $resin^{-1}$ d^{-1}) ($F_{10,164}$ = 1.3, P > 0.2; Table 3; Supplementary Online Materials Table S1F).

Resin-extractable NO_3^- flux showed different patterns from NH_4^+ flux, with significant effects of habitat, topography, and habitat by season but not of season alone ($F_{10,164}$ = 39.0, P < 0.0001; Figure 2c; Table 3; Supplementary Online Materials Table S1G). Habitat accounted for the largest proportion of the variance (61.5 %), followed by topography (4.7 %), and habitat by season (1.9 %). Premontane forest showed the highest NO_3^- flux (22.6 ± 2.0 µg N-NO_3 g $resin^{-1}$ d^{-1}), followed by lower montane forest (10.0 ± 1.2 µg N-NO_3 g $resin^{-1}$ d^{-1}) (Fisher's LSD, P < 0.05; Figure 2c). Upper montane forest (1.1 ± 0.2 µg N-NO_3 g $resin^{-1}$ d^{-1}) and montane grassland (1.7 ± 0.3 µg N-NO_3 g $resin^{-1}$ d^{-1}) showed significantly lower NO_3^- flux than the other two habitats (Fisher's LSD, P < 0.05; Figure 2c), with values that were not significantly different from each other (Fisher's LSD, P > 0.05; Figure 2c). For the effect of topography, multiple comparisons tests indicated that flat landforms (12.1 ± 1.8 µg N-NO_3 g $resin^{-1}$ d^{-1}) and slope landforms (10.2 ± 1.6 µg N-NO_3 g $resin^{-1}$ d^{-1}) differed significantly from ridge landforms (6.6 ± 1.4 µg N-NO_3 g $resin^{-1}$ d^{-1}) (Fisher's LSD, P < 0.05). The basin landform (3.8 ± 1.3 µg N-NO_3 g $resin^{-1}$ d^{-1}), despite the lower mean values, showed an overlapping range with the other landforms (Fisher's LSD, P > 0.05). The habitat by season interaction was due to the fact that upper montane forest shows a significant seasonal fluctuation in resin-extractable NO_3^- (Fisher's LSD, P < 0.05), whereas the other habitats show no significant seasonal trend (Fisher's LSD, P > 0.05; Table 3).

5.3 Effects of environmental variables on N_2O flux

For the whole dataset, the relationship between N_2O flux and environmental variables was examined using an ANCOVA on Box-Cox transformed data with habitat, season, topography, and environmental variables as covariates. Environmental variables included WFPS, oxygen, air temperature, soil temperature, and resin-extractable inorganic N flux (NH_4^+ and NO_3^-). The ANCOVA model as a whole was not statistically significant (P > 0.4). However, we found that individual factors were weakly but significantly correlated with N_2O flux for the pooled

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dataset. These included soil temperature (r^2= 0.04, P <0.0004), air temperature (r^2= 0.04, P <0.0008), and resin-extractable NO_3^- flux (r^2= 0.03, P <0.03). Water-filled pore space also showed a very weak negative correlation with N_2O flux at the borderline of statistical significance (r^2= 0.01, P <0.06).

For individual habitats, we explored how variations in environmental conditions influenced N_2O flux using multiple regression, with WFPS, oxygen, soil temperature, air temperature, resin-extractable NH_4^+ flux, and resin-extractable NO_3^- flux as explanatory variables. Only the multiple regression analysis for lower montane forest showed a borderline significant result, though only at the P < 0.07 level (r^2 = 0.36). The multiple regression models for all the other habitats were not statistically significant (P > 0.4). Lower montane forest was the only habitat that showed a significant effect of season on N_2O flux (section 5.1), and our multiple regression model corroborated this result by showing that seasonal fluctuations in air temperature, soil temperature, WFPS (Figure 3b), and NH_4^+ all correlated with N_2O flux (P < 0.05). Air temperature explained the largest proportion of variance in the data (26.2 %; negative trend), followed by soil temperature (15.5 %; positive trend), WFPS (13.7 %; negative trend), and resin-extractable NH_4^+ flux (11.6 %; negative trend).

5.4 Water-filled pore space manipulation

^{15}N-N_2O and ^{15}N-N_2 fluxes showed a biphasic response (Limmer and Steele, 1982), with significantly different flux rates in the first 24 hours of incubation compared to the later period of incubation (i.e. 24-48 hours). Flux of ^{15}N-N_2O, and ^{15}N-N_2 were therefore divided into early (0-24 hours) and late (24-48 hours) phase flux.

5.4.1 Role of nitrification and nitrate reduction in N_2O production

The ^{15}N flux data indicates that nitrate reduction (i.e. denitrification) was the dominant source of N_2O from these soils, while nitrification was only a minor contributor to ^{15}N-N_2O production (Supplementary Online Materials Figure S1). The ^{15}N-N_2O and ^{15}N-N_2 fluxes were analyzed using a full factorial ANOVA on Box-Cox transformed data with habitat, moisture level, form of ^{15}N-label added (i.e. $^{15}NH_4^{15}NO_3$ or $^{14}NH_4^{15}NO_3$), incubation phase, and all their interaction terms as independent variables. Notably, this analysis revealed that the form of ^{15}N-label added (i.e. ^{15}N-$NH_4^{15}N$-NO_3 or ^{14}N-$NH_4^{15}N$-NO_3) did not significantly alter ^{15}N-N_2O

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flux, indicating that production of ^{15}N-N_2O from nitrification was weak to negligible (Supplementary Online Materials Figure S1). In order to simplify our statistical analyses, all subsequent analyses were performed using only habitat, moisture level, incubation phase, and their interaction terms as independent variables. For these tests, which are described below, the "total" flux of ^{15}N-N_2O or ^{15}N-N_2 represents gas produced by both nitrification and nitrate reduction.

5.4.2 ^{15}N-N_2O flux

For the total ^{15}N-N_2O flux data, we used a full factorial ANOVA on Box-Cox transformed data with habitat, moisture level, incubation phase, and all their interactions as independent variables. We found that moisture level, habitat by incubation phase, and habitat by moisture by incubation phase were significantly related to ^{15}N-N_2O flux (ANOVA, $F_{31, 321}$ = 3.06, P < 0.0001; Figure 4; Supplementary Online Materials Table S2A). Of the three main factors (i.e. habitat, moisture level, incubation phase), moisture level was the dominant control on ^{15}N-N_2O flux (Supplementary Online Materials Table S2A). The highest ^{15}N-N_2O flux was observed in the 90 % WFPS (42 ± 9 ng N_2O-^{15}N g^{-1} d^{-1}) and 50 % WFPS (29 ± 10 ng N_2O-^{15}N g^{-1} d^{-1}) treatments, and the lowest flux in the 30 % (3 ± 1 ng N_2O-^{15}N g^{-1} d^{-1}) and 70 % (7 ± 2 ng N_2O-^{15}N g^{-1} d^{-1}) treatments (Fisher's LSD, P < 0.05; Figure 4). The habitat by incubation phase interaction indicated that some habitats showed different flux rates during early and late phases of the incubation (Figure 4). Premontane and lower montane forest showed statistically similar ^{15}N-N_2O flux during early and late incubation phases. Upper montane forest mineral layer soils showed a significant increase in ^{15}N-N_2O flux from early to late incubation phases (5 ± 2 ng N_2O-^{15}N g^{-1} d^{-1} versus 42 ± 13 ng N_2O-^{15}N g^{-1} d^{-}1; t-Test, P < 0.003), while montane grasslands showed a significant decrease in ^{15}N-N_2O flux from early to late incubation phases (60 ± 23 ng N_2O-^{15}N g^{-1} d^{-1} versus 6 ± 9 ng N_2O-^{15}N g^{-1} d^{-1}, respectively; t-Test, P < 0.02). The habitat by moisture by incubation phase effect stems from complex and varying responses of soils from different habitats to differences in moisture level and incubation phase (Figure 4).

5.4.3 $^{15}N^{-}N_2$ flux

For the total ^{15}N-N_2 flux data, we used a full factorial ANOVA on Box-Cox transformed data with habitat, moisture level, incubation phase, and all their interactions as independent

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variables. We found that all of the main factors and their interaction terms were statistically significant (ANOVA, $F_{31, 317} = 14.20$, $P < 0.0001$; Supplementary Online Materials Table S2B). Of the three main factors, habitat was the dominant control on $^{15}N\text{-}N_2$ flux (Supplementary Online Materials Table S2B). Lower montane forest showed the highest $^{15}N\text{-}N_2$ flux (694 ± 83 ng N_2-^{15}N g^{-1} d^{-1}); premontane forest and upper montane forest mineral layer soil showed intermediate levels of flux (326 ± 53 and 171 ± 20 ng N_2-^{15}N g^{-1} d^{-1}, respectively); and montane grassland soil showed the lowest flux (123 ± 23 ng N_2O-^{15}N g^{-1} d^{-1}) (Fisher's LSD, $P < 0.05$; Figure 4). Moisture played a secondary role in regulating $^{15}N\text{-}N_2$ flux (Supplementary Online Materials Table S2B), with only the 90 % treatment had significantly higher flux than the other treatments (90 % WFPS treatment: 437 ± 77 ng N_2-^{15}N g^{-1} d^{-1}; pooled average for all other treatments: 294 ± 28 ng N_2-^{15}N g^{-1} d^{-1}) (Fisher's LSD, $P < 0.05$). Incubation phase was the least important control on $^{15}N\text{-}N_2$ flux, with slightly greater flux of $^{15}N\text{-}N_2$ during the late compared to the early phase of the incubations (373 ± 44 ng N_2-^{15}N g^{-1} d^{-1} versus 288 ± 37 ng N_2-^{15}N g^{-1} d^{-1}) (t-Test, $P < 0.07$). The habitat by moisture level interaction indicates that flux from different habitats showed varying moisture responses (Figure 4). For example, $^{15}N\text{-}N_2$ flux from premontane forest and upper montane forest mineral layer soil showed no responses to moisture. In contrast, for lower montane forest, flux was greatest for the 90 % WFPS treatment (1,365 ± 201 ng N_2-^{15}N g^{-1} d^{-1}), lowest for the 70 % WFPS treatment (257 ± 128 ng N_2-^{15}N g^{-1} d^{-1}), and at intermediate levels for the 30 and 50 % WFPS treatments (664 ± 131 and 492 ± 79 ng N_2-^{15}N g^{-1} d^{-1}, respectively) (Fisher's LSD, $P < 0.05$). The pattern for montane grassland was different again; here, only the 90 % WFPS treatment showed significantly greater flux (171 ± 32 ng N_2-^{15}N g^{-1} d^{-1}) compared to the other treatments (pooled average: 105 ± 29 ng N_2-^{15}N g^{-1} d^{-1}) (Fisher's LSD, $P < 0.05$).

5.4.4 N_2O Yield

For the N_2O yield, we used a full factorial ANOVA on Box-Cox transformed data with habitat, moisture level, incubation phase, and all their interactions as independent variables. We found that habitat, moisture level, habitat by moisture level, habitat by phase, and habitat by moisture level by phase significantly influenced N_2O yield (ANOVA, $F_{31, 313} = 9.85$, $P < 0.0001$; Supplementary Online Materials Table S2C). Of the three main factors, habitat was the best predictor of N_2O yield (Supplementary Online Materials Table S2C). N_2O yield was highest for the montane grassland (0.61 ± 0.06), lowest for lower montane forest (0.19 ±

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0.04), while premontane forest and upper montane forest mineral layer soil showed similar intermediate values (0.40 ± 0.05 and 0.42 ± 0.05, respectively) (Fisher's LSD, $P < 0.05$). Moisture level explained much less of the variance in the dataset (Supplementary Online Materials Table S2C); N_2O yield was highest for the 70 % WFPS treatment (0.51 ± 0.06), while the 30, 50 and 90 % WFPS treatments showed statistically similar values (0.35 ± 0.05, 0.39 ± 0.05, and 0.36 ± 0.05, respectively) (Fisher's LSD, $P < 0.05$). For the habitat by moisture level interaction, this reflects the fact that only lower montane forest and upper montane forest showed differences in N_2O yield with changes in moisture level. For the lower montane forest, N_2O yield was greatest in the 70 % WFPS treatment (0.51 ± 0.11), whereas the other treatments were not statistically different from each other (pooled average: 0.09 ± 0.03) (Fisher's LSD, $P < 0.05$). Upper montane forest mineral layer soil showed the highest N_2O yield for the 90 % treatment (0.72 ± 0.08), lowest yield for the 30 % WFPS treatment (0.20 ± 0.09), and intermediate N_2O yields for the 50 and 70 % WFPS treatments (0.29 ± 0.09 and 0.50 ± 0.11, respectively) (Fisher's LSD, $P < 0.05$). For the habitat by incubation phase interaction, this reflects the fact that upper montane forest mineral layer soil showed an increase in N_2O yield from early to late phase, while montane grassland showed a decrease in N_2O yield from early to late phase. The habitat by moisture level by incubation phase interaction reflects the complex and varied responses of soils from different habitats to changes in moisture level and incubation phase (Figure 4).

5.5 Litter manipulation experiment

In order to investigate the relationship between leaf litter input rates and N_2O flux, we used a Generalized Linear Model (GLM) and an ANCOVA that included habitat, litter treatment, season, WFPS, litter input rate, litter C input rate, litter N input rate, soil temperature and air temperature as independent variables. The analysis was also repeated using ANCOVA on Box-Cox transformed data. Both analyses revealed no significant statistical relationship between N_2O flux and any of these environmental variables, with the exception of soil temperature, which showed only a weak positive relationship to N_2O flux when the data was analysed using the GLM ($P < 0.05$). This relationship was not detected using ANCOVA. Bivariate regression of soil temperature against N_2O flux indicated that the relationship was relatively weak, with $r^2 = 0.01$ ($P < 0.05$).

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fact that the moisture response of different habitats was contingent upon incubation phase. For instance, for upper montane forest mineral layer soil, N_2O yield during the early phase was greatest for the 90 % WFPS treatment (1; i.e. no $^{15}N\text{-}N_2$ flux observed), while the 50 % WFPS treatment showed intermediate N_2O yield (0.33 ± 12), and the 30 and 70 % WFPS treatments collectively showed the lowest N_2O yields (approximately 0 for both; i.e. no $^{15}N\text{-}N_2O$ flux observed) (Fisher's LSD, $P < 0.05$). In contrast, during the late phase, the 70 % WFPS treatment showed the highest N_2O yield (1; i.e. no $^{15}N\text{-}N_2$ flux observed), while the other treatments showed lower N_2O yields that were not significantly different from each other (pooled average: 0.33 ± 0.07) (Fisher's LSD, $P < 0.05$). In contrast, for montane grassland, no effect of moisture was observed during the early phase of the incubation. However, during the late phase, the 50 % WFPS treatment showed the highest N_2O yield (0.89 ± 0.11), while the other treatments showed lower N_2O yields that were not significantly different from each other (pooled average: 0.39 ± 0.10) (Fisher's LSD, $P < 0.05$). For all other habitats with no habitat by phase interaction (i.e. premontane and lower montane forest), the moisture effect follows the general trends described above.

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fact that the moisture response of different habitats was contingent upon incubation phase. For instance, for upper montane forest mineral layer soil, N_2O yield during the early phase was greatest for the 90 % WFPS treatment (1; i.e. no $^{15}N\text{-}N_2$ flux observed), while the 50 % WFPS treatment showed intermediate N_2O yield (0.33 ± 12), and the 30 and 70 % WFPS treatments collectively showed the lowest N_2O yields (approximately 0 for both; i.e. no $^{15}N\text{-}N_2O$ flux observed) (Fisher's LSD, $P < 0.05$). In contrast, during the late phase, the 70 % WFPS treatment showed the highest N_2O yield (1; i.e. no $^{15}N\text{-}N_2$ flux observed), while the other treatments showed lower N_2O yields that were not significantly different from each other (pooled average: 0.33 ± 0.07) (Fisher's LSD, $P < 0.05$). In contrast, for montane grassland, no effect of moisture was observed during the early phase of the incubation. However, during the late phase, the 50 % WFPS treatment showed the highest N_2O yield (0.89 ± 0.11), while the other treatments showed ... [5]

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5.6 Nitrate addition experiment

^{15}N-N_2O and ^{15}N-N_2 fluxes showed a biphasic response (Limmer and Steele, 1982), with significantly different flux rates in the first 24 hours of incubation compared to the later period of incubation (i.e. 24-48 hours). Flux of ^{15}N-N_2O, and ^{15}N-N_2 were therefore divided into early (0-24 hours) and late (24-48 hours) phase flux.

5.6.1 ^{15}N-N_2O flux

For the ^{15}N-N_2O flux data, we used a full factorial ANOVA on Box-Cox transformed data with habitat, N addition level, incubation phase, and all their interaction terms as independent variables. Habitat, incubation phase, and the habitat by incubation phase interaction all significantly influenced ^{15}N-N_2O flux (ANOVA, $F_{29,\ 149} = 5.67$, $P < 0.0001$; Figure 5; Supplementary Online Materials Table S3A). Notably, N addition level did not significantly influence ^{15}N-N_2O flux. Of the three main factors (i.e. habitat, N addition level, incubation phase), habitat was the best predictor of ^{15}N-N_2O flux, explaining a largest proportion of the variance (Supplementary Online Materials Table S3A). Upper montane forest organic layer soils showed the highest flux (238 ± 160 ng N_2O-^{15}N g^{-1} d^{-1}), lower montane (179 ± 48 ng N_2O-^{15}N g^{-1} d^{-1}) and premontane (86 ± 16 ng N_2O-^{15}N g^{-1} d^{-1}) forest showed intermediate flux, while montane grasslands (11 ± 4 ng N_2O-^{15}N g^{-1} d^{-1}) and upper montane forest mineral layer soils (0.06 ± 0.01 ng N_2O-^{15}N g^{-1} d^{-1}) showed the lowest flux (Fisher's LSD, $P < 0.05$). The effect of incubation phase was attributable to significantly greater ^{15}N-N_2O flux during the late compared to early incubation phases (164 ± 66 ng N_2O-^{15}N g^{-1} d^{-1} versus 42 ± 11 ng N_2O-^{15}N g^{-1} d^{-1}; t-Test, $P < 0.05$; Figure 5). The habitat by incubation phase interaction was caused by some habitats showing higher flux in certain incubation phases than others (Figure 5). During the early phase, lower montane and premontane forests collectively showed the highest flux (Figure 5; Fisher's LSD, $P < 0.05$). In contrast, during the late incubation phase, upper montane forest organic layer soils, lower montane forest, and premontane forest now showed the highest flux (Figure 5; Fisher's LSD, $P < 0.05$).

5.6.2 ^{15}N-N_2 flux

For the ^{15}N-N_2 flux data, we used a full factorial ANOVA on Box-Cox transformed data with habitat, N addition level, incubation phase, and all their interaction terms as independent variables. Only habitat significantly influenced flux, while other terms were not significant

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(ANOVA, $F_{29, 149}$ = 1.66, P < 0.05; Figure 5; Supplementary Online Materials Table S3B). Lower montane and upper montane forest organic layer soils showed the highest flux (472 ± 139 and 576 ± 117 ng N_2-^{15}N g^{-1} d^{-1}, respectively), while all other habitats showed similar flux rates (105 ± 19 ng N_2-^{15}N g^{-1} d^{-1}) (Fisher's LSD, P < 0.05; Figure 5).

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5.6.3 N_2O Yield

For the N_2O yield, we used a full factorial ANOVA on Box-Cox transformed data with habitat, N addition level, incubation phase (i.e. early versus late), and all their interaction terms as independent variables. We found that none of these factors predicted N_2O yield (ANOVA, $F_{29, 149}$ = 0.75, P > 0.82; Supplementary Online Materials Table S3C). The overall mean N_2O yield for the pooled dataset was 0.53 ± 0.04.

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6. Discussion

6.1 Effects of seasonality and soil moisture on N_2O flux

Nitrous oxide flux in the Kosñipata Valley showed weak seasonality, with greater N_2O flux during the dry season compared to the wet season. This regional trend was consistent with results from our prior study, and was principally driven by strong seasonality in N_2O flux from lower montane forest (Teh et al., 2014). In contrast, other habitats showed little or no seasonal variation in N_2O flux. This weak seasonality in N_2O flux across the Kosñipata Valley probably stems from relatively modest variation in environmental variables among seasons (Table 3), in accordance with observations from elsewhere in the Andes (Baldos et al., 2015;Müller et al., 2015;Wolf et al., 2011). For example, while soil moisture (i.e. WFPS) varied significantly between seasons in the dataset as a whole, the absolute difference in WFPS between dry season and wet season were relatively small (i.e. 7.4 %). Indeed, some habitats showed much smaller variations in soil moisture, such as premontane forest and montane grassland that showed no significant seasonal variation in WFPS whatsoever (Table 3).

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One critical factor contributing to these weak seasonal trends in N_2O flux is the atypical response of N_2O flux to changes in soil moisture. Nitrous oxide flux showed a weak but negative correlation with WFPS in the field dataset (r^2= 0.01, P <0.06 for the pooled dataset),

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rather than following a curvilinear pattern predicted by denitrification theory (Firestone and Davidson, 1989;Firestone et al., 1980;Weier et al., 1993;Davidson, 1991). Likewise, in our soil moisture manipulation experiments, nitrification made a minor contribution to N_2O production, irrespective of soil moisture content (Supplementary Online Materials Figure S1). This finding is contrary to theoretical predictions of N_2O production by ammonia-oxidizing bacteria (AOB), where N_2O production from ammonia-oxidation is thought to make an important contribution to N_2O flux at lower soil moisture contents (i.e. 30-60 % WFPS) (Firestone and Davidson, 1989;Firestone et al., 1980;Weier et al., 1993;Davidson, 1991). At higher soil moisture contents (i.e. >60 % WFPS), N_2O flux showed a non-linear response to increasing WFPS, with two distinct peaks in N_2O flux at 90 and 50 % WFPS (Figure 4). Collectively, these findings suggest that the role of soil moisture in regulating N_2O flux is more complex than predicted by existing theory, falsifying our first two hypotheses.

What could explain these unexpected trends? We believe that these patterns occurred due to the complex interplay between environmental conditions and the microbial processes that produce N_2O in soil (i.e. ammonia oxidation by archaea, ammonia oxidation by bacteria, denitrification, dissimilatory nitrate reduction to ammonium). We suspect that the action of lesser-known microbial processes, such as oxidation of ammonia by archaea and dissimilatory nitrate reduction to ammonium (DNRA), may explain the divergence from theoretical norms. Our expectations of how N_2O production should respond to variations in soil moisture are predicated on the assumption that N_2O is produced almost exclusively by AOB and denitrifying bacteria, with the former operating at lower soil moisture content (i.e. 30-60 % WFPS) and the latter at higher soil moisture content (i.e. >60 % WFPS) (Firestone and Davidson, 1989;Firestone et al., 1980;Weier et al., 1993;Davidson, 1991). More recent advances in soil N research, however, have highlighted the importance of other microbial taxa or processes, not previously considered in conceptual or process-based models. For example, recent work in acidic soils have demonstrated that ammonia oxidizing archaea (AOA) play a more important role than AOB in ammonia oxidation, but produce significantly less N_2O due to differences in metabolism (Hink et al., 2016;Prosser and Nicol, 2008). Likewise, under higher soil moisture conditions (>60 % WFPS), DNRA – a process that produces substantially less N_2O than denitrification and which also competes for NO_3^- with denitrification – can dominate nitrate reduction, depending on redox conditions and the

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relative availability of labile C and N (Morley and Baggs, 2010;Pett-Ridge and Firestone, 2005;Silver et al., 2001;Baldos et al., 2015;Müller et al., 2015). Thus, given the low pH of the soils in Kosñipata Valley (Table 1), it is likely that AOA dominate ammonia oxidation at lower levels of soil moisture, explaining the negligible amounts of N_2O produced from nitrification in the 30 and 50 % WFPS treatments. As soils become wetter, the non-linear response of N_2O flux to increasing soil moisture may reflect competition for substrates (e.g. NO_3^-, reducing equivalents) between DNRA and denitrification (Morley and Baggs, 2010;Silver et al., 2001), or may indicate that DNRA is making a larger contribution to N_2O flux than denitrification (Streminska et al., 2012).

These findings are important and noteworthy, given that climatically-driven variations in soil moisture content are thought to be one of the dominant drivers for N_2O flux in the seasonally dry tropics (Davidson, 1991;Firestone and Davidson, 1989;Groffman et al., 2009;Davidson and Verchot, 2000;Teh et al., 2014;van Lent et al., 2015;Werner et al., 2007). Moreover, similar results from comparable research sites in the Ecuadorian Andes lend credence to our claims (Baldos et al., 2015;Müller et al., 2015). For example, Müller et al. (2015) found that nitrification produced little or no N_2O in acidic Ecuadorian soils, in agreement with findings from in this study. Likewise, ^{15}N isotope pool dilution experiments, in comparable habitats and elevations to our own, revealed that DNRA played a significant role in nitrate reduction, supporting the notion that DNRA may represent a substantial sink for NO_3^- in Peruvian soils (Baldos et al., 2015;Müller et al., 2015). Existing process-based models, which are used to construct bottom-up emissions inventories for the tropics (Werner et al., 2007), often assume that N_2O is derived primarily from AOB and denitrification, with moisture response curves based on existing theoretical relationships (Li et al., 2000;Werner et al., 2007;Smith et al., 2007). However, if these more "normative" soil moisture response curves are inapplicable to montane tropical ecosystems, due to the activity of AOA and DNRA, then a re-conceptualisation of the soil moisture-N_2O flux relationship may be required. Moreover, if weak seasonality or aseasonality in N_2O flux is the norm in Andean ecosystems (Müller et al., 2015;Wolf et al., 2011), then this finding may have wider implications for understanding spatial or temporal trends in regional atmospheric budgets (Kort et al., 2011;Nevison et al., 2004;Nevison et al., 2007;Saikawa et al., 2014).

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6.2 Role of substrate limitation in regulating N_2O flux

In accordance with our earlier work (Teh et al., 2014) and research conducted in analogous ecosystems in Ecuador (Baldos et al., 2015;Müller et al., 2015;Wolf et al., 2011), we found strong evidence that N_2O flux was constrained by the availability of NO_3^-, partially supporting our third hypothesis. In contrast, N_2O flux was unresponsive to short-term changes in labile organic matter (i.e. leaf litter-fall) inputs, indicating that N_2O flux and nitrate reduction were not C limited. This latter result is significant for modelling and extrapolating N_2O flux from these habitats, because many process-based models assume that N cycling and turnover of labile organic matter are intimately linked through processes such as litter production and decomposition (Li et al., 2000;Werner et al., 2007;Smith et al., 2007).

Evidence for NO_3^- limitation of N_2O flux comes from both our field and laboratory data, and suggests that "habitat" may be a good proxy for NO_3^- availability and N_2O flux because these two variables co-vary with habitat. For example, we observed an inverse trend in field N_2O flux, with premontane forest showing significantly greater flux than the other habitats elevation (Table 3, Figure 2a). This inverse trend was also reflected in the resin-extractable NO_3^- flux measured in the field and the ^{15}N-N_2O flux measured in the NO_3^- addition experiment in the laboratory (Figure 2c, 5a). Furthermore, the behaviour of the ^{15}N-NO_3^- amended soils during the early (≤24 hours) and late (>24 hours) phases of the incubation experiment suggest that soils from more N-poor habitats (i.e. those with lower rates of resin-extractable NO_3^- flux; Table 3, Figure 2c) showed a greater proportional increase in ^{15}N-N_2O flux following NO_3^- addition than N-rich habitats (i.e. those with higher rates of resin-extractable NO_3^- flux; Table 3, Figure 2c), suggesting that ^{15}N-N_2O flux was more NO_3^- limited in N-poor soils (Figure 5). Soils from the upper montane forest organic layer, montane grasslands, and upper montane forest mineral layer showed the lowest ^{15}N-N_2O flux during the early phase of soil incubation, but the greatest proportional increase in flux during the late phase of soil incubation, rising by a factor of 59, five, and two, respectively. In contrast, lower montane and premontane forest soils showed the smallest proportional increase in the late phase of soil incubation (i.e. 1.7 times increase). Last, the relatively low N_2O yield observed in our soil moisture manipulations is thought to be broadly indicative of low NO_3^- conditions (i.e. <0.42 for forested habitats; Table 4), further supporting the notion that N_2O

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flux in this region is generally NO_3^- limited (Schlesinger, 2009;Fang et al., 2015;Weier et al., 1993).

Interestingly, we found no evidence that these soils responded to short-term increases in NO_3^- availability, at least within the concentration range used for the experiments described here. $^{15}N\text{-}N_2O$ flux, $^{15}N\text{-}N_2$ flux, and N_2O yield were not directly influenced by the amount of $^{15}N\text{-}NO_3^-$ added (Figure 5). Rather, ANCOVA suggests that $^{15}N\text{-}N_2O$ and $^{15}N\text{-}N_2$ fluxes were better-predicted by habitat. N_2O yield, normally a sensitive indicator of NO_3^- availability (Blackmer and Bremner, 1978;Weier et al., 1993;Parton et al., 1996), showed no immediate response to the amount of $^{15}N\text{-}NO_3^-$ added, nor any of the other explanatory variables. One explanation for this, consistent with the notion that N_2O flux is NO_3^- limited, is that nitrate-reducing microbes in these soils may have a relatively low half-saturation constant (K_m) for NO_3^-, and effectively utilize NO_3^- whenever concentrations increase above baseline (i.e. non-limiting) levels (Holtan-Hartwig et al., 2000). As a consequence, we may be unable to differentiate among NO_3^- treatments because the NO_3^- addition levels that we used all exceeded the K_m for these soils. This finding is also in agreement with results from long-term N fertilization studies, which suggest that substantive shifts in N_2O flux are only likely to occur after prolonged exposure to high levels of N (i.e. >1 year), rather than due to transient fluctuations in N availability (Baldos et al., 2015;Corre et al., 2010;Müller et al., 2015;Hall and Matson, 1999;Koehler et al., 2012).

6.3 Implications for annual atmospheric budgets and gaseous N loss

Montane ecosystems in the Kosñipata Valley were net sources of atmospheric N_2O, affirming our prior results (Teh et al., 2014). The flux for this multi-annual dataset was comparable to the preliminary values reported in our earlier publication, with an unweighted mean flux of 0.27 ± 0.07 mg $N\text{-}N_2O$ m^{-2} d^{-1} observed over a 30 month period compared to 0.22 ± 0.12 mg $N\text{-}N_2O$ m^{-2} d^{-1} recorded over a 13 month period (Teh et al., 2014). These values correspond to unweighted mean annual fluxes of 0.99 ± 0.26 kg $N_2O\text{-}N$ ha^{-1} $year^{-1}$ and 0.80 ± 0.44 kg $N_2O\text{-}N$ ha^{-1} $year^{-1}$, respectively. However, in order to derive more accurate estimates of the annual contribution of the Kosñipata Valley to the regional atmospheric budget of N_2O, it is necessary to account for differences in land area for different habitats and variation in the magnitude of N_2O flux between seasons. Thus, we conducted a simple weighted upscaling

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exercise to more fully account for these two sources of variation (Table 4). Using the N_2O yield data from the laboratory tracer experiments, we also estimated the annual N_2 flux and total gaseous N flux, in order compare rates of gaseous N export from this region with other forested ecosystems (Fang et al., 2015;Russell and Raich, 2012;Tietema and Verstraten, 1991;Bai et al., 2012) (Table 4). We fully acknowledge that this simple approach is not as robust as bottom-up, process-based emissions inventories (Werner et al., 2007). Even so, we believe it is still useful for providing first-order approximations of annual N_2O, N_2 and total gaseous N flux.

To briefly summarize our methodology, our first step was to use published surface area estimates for the different habitats in the Kosñipata Valley to derive areal fractions for each habitat (Feeley and Silman, 2010) (Table 4). Next, we multiplied the unweighted seasonal mean flux by the areal fraction for each habitat to derive area-weighted seasonal flux estimates (Table 4). We subsequently multiplied the area-weighted seasonal flux by the fraction of the year accounted for by either season, in order to produce an area-weighted and seasonally-weighted annual flux estimate for each habitat (Table 4). The final step of this process was to sum the area-weighted and seasonally-weighted flux estimates for each habitat, to drive an overall weighted flux estimate for the Kosñipata Valley as a whole (Table 4). Weighted annual estimates of N_2 flux were calculated using the N_2O yield values for each habitat as determined in our soil moisture manipulation experiment (Table 4). We elected to use mean N_2O yields for each habitat, rather than estimating N_2O yield based on soil moisture content, because ANCOVA indicated that habitat was a better predictor of N_2O yield than soil moisture, explaining a substantially greater proportion of the variance (i.e. 10 % versus only 1 % of the variance; see Supplementary Online Materials Table S2C). Total gaseous N export was estimate by calculating the sum of annual N_2O and N_2 flux. Errors for all the annual flux estimates (i.e. N_2O, N_2, total gaseous N) were propagated using standard error propagation techniques.

We determined that the Kosñipata Valley emitted approximately 1.27 ± 0.33 kg N_2O-N ha^{-1} $year^{-1}$, 3.29± 1.27 kg N_2-N ha^{-1} $year^{-1}$, and 4.57 ± 1.31 kg N ha^{-1} $year^{-1}$. Annual N_2O flux was broadly on par with our earlier estimates (i.e. 1.18 ± 0.79 kg N_2O-N ha^{-1} $year^{-1}$) (Teh et al., 2014). This estimated annual rate of flux exceeds the value for montane tropical montane

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forests calculated by Werner et al. (2007) using a bottom-up process model (i.e. 0.5 to 1 kg N_2O-N ha^{-1} $year^{-1}$), but falls within the range predicted for humid tropical forest soils more generally (i.e. approximately 1-4 kg N_2O-N ha^{-1} $year^{-1}$) (van Lent et al., 2015;Werner et al., 2007). Annual N_2 flux and total gaseous N flux are at the lower end of the range reported in comparable studies from other ecosystems (e.g. Fang et al., 2015 reported annual gaseous losses of 5.6– 30.1 kg N ha^{-1} $year^{-1}$ sampling across a broad range of temperate and tropical ecosystems) (Fang et al., 2015;Russell and Raich, 2012;Tietema and Verstraten, 1991;Bai et al., 2012), further supporting claims that Andean ecosystems are relatively N limited, and may cycle N more conservatively than lowland forests (Baldos et al., 2015;Müller et al., 2015;Wolf et al., 2011;Nottingham et al., 2015)

7. Conclusions

Process-based studies of N_2O flux from montane tropical ecosystems in the southern Peruvian Andes affirms prior research suggesting that these ecosystems are potentially important regional sources of N_2O (Teh et al., 2014). Simple weighted upscaling suggests that annual N_2O flux from the Kosñipata Valley is on the order of 1.27 ± 0.33 kg N_2O-N ha^{-1}. Habitat – a proxy for NO_3^- availability under field conditions – was the best predictor for N_2O flux, with more N-rich habitats (i.e. premontane forest) showing significantly higher N_2O flux than habitats with lower N availability (i.e. upper montane forest, montane grassland). Nitrous oxide flux originated primarily from nitrate reduction rather than from nitrification, probably due to low pH soil conditions which may have inhibited the activity of AOB. Contrary to our prior research, we found only weak evidence for seasonal trends in field N_2O flux, with the exception of lower montane forest, which showed significantly higher N_2O flux during the dry season compared to the wet season. Weak seasonal trends in field N_2O flux among the other montane habitats probably stems from relatively modest seasonal variation in key environmental drivers (e.g. temperature, WFPS, NO_3^-), combined with a soil moisture response that was complex and non-linear. Nitrous oxide flux was significantly influenced by soil moisture content, but the trends in N_2O production and flux diverged from theoretical norms. For example, we saw little evidence of N_2O production from ammonia-oxidation, even though the field measurement (i.e. resin bags) indicate that nitrification occurs. This may be due to the predominance of AOA, which produce significantly N_2O than

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AOB, under the acidic conditions common in Andean soils. At higher soil moisture levels, N_2O flux increased non-linearly with WFPS, with peaks in N_2O flux at 90 and 50 % WFPS. These results suggest that the effects of water on N_2O flux are complicated by other factors, such as competition for substrates among different nitrate-reducing processes, or shifts in the amount of N_2O derived from denitrification or DNRA. Field data and substrate manipulation experiments indicated that N_2O flux was strongly limited by NO_3^-, but unconstrained by the input rate of labile organic matter (i.e. leaf litter). Nitrous oxide flux was relatively insensitive to short-term variations in NO_3^-, and was better-predicted by longer-term, time-averaged variations in NO_3^- availability.

8. Data Availability

Data for this publication are publically available from the UK Natural Environment Research Council (NERC) Centre for Environmental Data Analysis (CEDA), at the following URL: http://catalogue.ceda.ac.uk/uuid/93fdb48b713b4dbc93a28d695771312d

9. Author Contributions

TD designed the field and laboratory experiments, collected the field data, conducted the laboratory experiments, processed the samples, analysed the data, and contributed to the preparation of the manuscript. NJM contributed to the design of the laboratory experiments, assisted in the sample analysis, assisted in the analysis of the laboratory data, and contributed to the preparation of the manuscript. AJC and LPHQ assisted in the collection of the field data and processing of the field samples. EMB, PM, MR, and PS contributed to the experimental design and the preparation of the manuscript. YAT directed the research, contributed to the design of the experiments, assisted in the analysis of the field and laboratory data, and took the principal role in preparing the manuscript.

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NE/H007849 and NE/H006753). Patrick Meir was supported by an Australian Research Council Fellowship (FT110100457). Javier Eduardo Silva Espejo, Walter Huaraca Huasco, and the ABIDA NGO provided critical fieldwork and logistical support. Angus Calder (University of St Andrews) and Vicky Munro (University of Aberdeen) provided invaluable laboratory support. Thanks to Adrian Tejedor from the Amazon Conservation Association, who provided assistance with site access and site selection at Hacienda Villa Carmen. This publication is a contribution from the Scottish Alliance for Geoscience, Environment and Society (http://www.sages.ac.uk).

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12. Tables and Figures

Table 1. Site characteristics.

Elevation Band m a.s.l.	Habitat	Latitude	Longitude	Mean Annual Temperature °C	Mean Annual Precipitation mm	Bulk density 0-10 cm g cm-3	pH	Soil C:N 0-10 cm	Soil C 0-10 cm %	Mineral Soil Particle Size 0-10 cm Clay	0-10 cm Silt	0-10 cm Sand	10-30 cm Clay	10-30 cm Silt	10-30 cm Sand	Landforms	Plots n	Flux Chambers n
600-1200	Premontane forest	12°53'43"	71°23'04"	20.5	5318	0.38 ± 0.03 (n = 21)	3.4 ± 0.1	11.3 ± 0.2	7.9 ± 0.5	5.4 ± 0.3	68.8 ± 3.9	25.4 ± 15.9	8.9 ± 1.8	81.0 ± 1.7	10.3 ± 2.5	ridge, slope, flat	3	15
1200-2200	Lower montane forest	13°2'56"	71°32'13"	17.2	2631	0.19 ± 0.03 (n = 17)	3.4 ± 0.1	14.5 ± 0.2	25.2 ± 1.3	3.6 ± 0.4	67.3 ± 4.2	29.3 ± 4.5	7.2 ± 0.4	83.8 ± 0.8	9.0 ± 0.9	ridge, slope, flat	3	15
2200-3200	Upper montane forest	13°11'24"	71°35'13"	10.7	1706	0.41 ± 0.02 (n = 12)	3.9 ± 0.1	16.8 ± 0.4	16.3 ± 1.0	5.1 ± 0.9	57.1 ± 7.9	37.9 ± 8.7	4.4 ± 2.0	46.5 ± 16.2	49.1 ± 18.1	ridge, slope	3	15
3200-3700	Montane grassland	13°07'19"	71°36'54"	9.3	2200	0.36 ± 0.03 (n = 27)	4.1 ± 0.1	12.9 ± 0.4	16.0 ± 1.0	2.6 ± 0.2	54.4 ± 3.0	43.0 ± 3.2	n/a	n/a	n/a	ridge, slope, flat, basin	4	20

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Table 2. Description of the water-filled pore space and NO_3^- addition treatments for the laboratory manipulation experiments.

Habitat	Experimental Treatment	Soil Depth	Soil Type	WFPS	Inorganic N added		Replicate
				%	ng N (g soil)$^{-1}$	^{15}N Tracer	*n*
WATER-FILLED PORE SPACE							
Premontane forest	90 % WFPS	0-10	mineral	90	200	$^{15}NH_4^{15}NO_3$	5
	90 % WFPS	0-10	mineral	90	200	$^{14}NH_4^{15}NO_3$	5
	70 % WFPS	0-10	mineral	70	200	$^{15}NH_4^{15}NO_3$	5
	70 % WFPS	0-10	mineral	70	200	$^{14}NH_4^{15}NO_3$	5
	50 % WFPS	0-10	mineral	50	200	$^{15}NH_4^{15}NO_3$	5
	50 % WFPS	0-10	mineral	50	200	$^{14}NH_4^{15}NO_3$	5
	30 % WFPS	0-10	mineral	30	200	$^{15}NH_4^{15}NO_3$	5
	30 % WFPS	0-10	mineral	30	200	$^{14}NH_4^{15}NO_3$	5
Lower montane forest	90 % WFPS	0-10	mineral	90	200	$^{15}NH_4^{15}NO_3$	5
	90 % WFPS	0-10	mineral	90	200	$^{14}NH_4^{15}NO_3$	5
	70 % WFPS	0-10	mineral	70	200	$^{15}NH_4^{15}NO_3$	5
	70 % WFPS	0-10	mineral	70	200	$^{14}NH_4^{15}NO_3$	5
	50 % WFPS	0-10	mineral	50	200	$^{15}NH_4^{15}NO_3$	5
	50 % WFPS	0-10	mineral	50	200	$^{14}NH_4^{15}NO_3$	5
	30 % WFPS	0-10	mineral	30	200	$^{15}NH_4^{15}NO_3$	5
	30 % WFPS	0-10	mineral	30	200	$^{14}NH_4^{15}NO_3$	5
Upper montane forest	90 % WFPS	10-20	mineral	90	20	$^{15}NH_4^{15}NO_3$	5
	90 % WFPS	10-20	mineral	90	20	$^{14}NH_4^{15}NO_3$	5
	70 % WFPS	10-20	mineral	70	20	$^{15}NH_4^{15}NO_3$	5
	70 % WFPS	10-20	mineral	70	20	$^{14}NH_4^{15}NO_3$	5
	50 % WFPS	10-20	mineral	50	20	$^{15}NH_4^{15}NO_3$	5
	50 % WFPS	10-20	mineral	50	20	$^{14}NH_4^{15}NO_3$	5
	30 % WFPS	10-20	mineral	30	20	$^{15}NH_4^{15}NO_3$	5
	30 % WFPS	10-20	mineral	30	20	$^{14}NH_4^{15}NO_3$	5
Montane grassland	90 % WFPS	0-10	mineral	90	20	$^{15}NH_4^{15}NO_3$	5
	90 % WFPS	0-10	mineral	90	20	$^{14}NH_4^{15}NO_3$	5
	70 % WFPS	0-10	mineral	70	20	$^{15}NH_4^{15}NO_3$	5
	70 % WFPS	0-10	mineral	70	20	$^{14}NH_4^{15}NO_3$	5
	50 % WFPS	0-10	mineral	50	20	$^{15}NH_4^{15}NO_3$	5
	50 % WFPS	0-10	mineral	50	20	$^{14}NH_4^{15}NO_3$	5
	30 % WFPS	0-10	mineral	30	20	$^{15}NH_4^{15}NO_3$	5
	30 % WFPS	0-10	mineral	30	20	$^{14}NH_4^{15}NO_3$	5
NITRATE ADDITION							
Premontane forest	control	0-10	mineral	80	n/a	n/a	5
	+50 % background NO_3^-	0-10	mineral	80	780 ± 60	$K^{15}NO_3$	5
	+100 % background NO_3^-	0-10	mineral	80	1570 ± 120	$K^{15}NO_3$	5
	+150 % background NO_3^-	0-10	mineral	80	2350 ± 170	$K^{15}NO_3$	5
Lower montane forest	control	0-10	mineral	80	n/a	n/a	5
	+50 % background NO_3^-	0-10	mineral	80	700 ± 60	$K^{15}NO_3$	5
	+100 % background NO_3^-	0-10	mineral	80	1400 ± 120	$K^{15}NO_3$	5
	+150 % background NO_3^-	0-10	mineral	80	2100 ± 180	$K^{15}NO_3$	5
Upper montane forest	control	0-10	organic	80	n/a	n/a	5
	+50 % background NO_3^-	0-10	organic	80	90 ± 20	$K^{15}NO_3$	5
	+100 % background NO_3^-	0-10	organic	80	180 ± 50	$K^{15}NO_3$	5
	+150 % background NO_3^-	0-10	organic	80	270 ± 70	$K^{15}NO_3$	5
	control	10-20	mineral	80	n/a	n/a	5
	+50 % background NO_3^-	10-20	mineral	80	90 ± 40	$K^{15}NO_3$	5
	+100 % background NO_3^-	10-20	mineral	80	190 ± 70	$K^{15}NO_3$	5
	+150 % background NO_3^-	10-20	mineral	80	280 ± 110	$K^{15}NO_3$	5
Montane grassland	control	0-10	mineral	80	n/a	n/a	5
	+50 % background NO_3^-	0-10	mineral	80	30 ± 10	$K^{15}NO_3$	5
	+100 % background NO_3^-	0-10	mineral	80	60 ± 20	$K^{15}NO_3$	5
	+150 % background NO_3^-	0-10	mineral	80	90 ± 40	$K^{15}NO_3$	5

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Table 3. Seasonal patterns in net N_2O flux, net inorganic N flux, and environmental variables, Lower case letters indicate difference among seasons within habitats (*t*-Test on Box-Cox transformed data, $P < 0.05$). Values reported here are means and standard errors.

Habitat	N_2O mg N-N_2O m^{-2} d^{-1}		WFPS %		Soil Temperature °C		Air Temperature °C		Oxygen %		NO_3^- µg N-NO_3^- (g resin)$^{-1}$ d^{-1}		NH_4^+ µg N-NH_4^+ (g resin)$^{-1}$ d^{-1}	
	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season
Premontane	0.71 ± 0.25 a n = 130	0.79 ± 0.26 a n = 98	51.9 ± 1.6 a n = 135	51.2 ± 2.1 a n = 135	20.7 ± 0.1 a n = 143	20.2 ± 0.1 b n = 120	21.5 ± 0.3 n = 143	20.4 ± 0.5 n = 120	19.4 ± 0.2 a n = 52	19.6 ± 0.2 a n = 36	23.2 ± 3.6 a n = 89	22.1 ± 2.1 a n = 96	31.4 ± 13.0 n = 90	11.3 ± 1.8 n = 95
Lower montane	0.09 ± 0.08 a n = 212	1.02 ± 0.58 b n = 137	42.2 ± 1.0 a n = 271	34.0 ± 1.4 b n = 179	18.1 ± 0.1 a n = 254	17.3 ± 0.2 b n = 164	18.9 ± 0.3 n = 254	18.3 ± 0.2 n = 164	19.2 ± 0.2 a n = 146	19.2 ± 0.1 a n = 81	11.8 ± 1.9 a n = 123	7.8 ± 1.4 a n = 94	20.2 ± 5.4 n = 124	8.6 ± 0.9 n = 93
Upper montane	0.06 ± 0.09 a n = 207	0.01 ± 0.11 a n = 146	42.0 ± 1.3 a n = 264	24.3 ± 1.4 b n = 180	11.8 ± 0.1 a n = 255	10.9 ± 0.2 b n = 165	12.8 ± 0.2 n = 255	12.5 ± 0.3 n = 165	18.7 ± 0.2 a n = 165	18.5 ± 0.2 a n = 109	1.4 ± 0.2 a n = 128	0.6 ± 0.2 b n = 91	22.5 ± 6.3 n = 129	11.3 ± 1.4 n = 93
Montane grassland	-0.01 ± 0.11 a n = 238	0.19 ± 0.12 a n = 160	88.5 ± 0.3 a n = 303	88.3 ± 0.5 a n = 184	11.6 ± 0.1 a n = 282	9.0 ± 0.2 b n = 205	11.4 ± 0.3 n = 284	12.0 ± 0.5 n = 205	12.2 ± 0.9 a n = 176	15.4 ± 0.8 b n = 117	1.5 ± 0.4 a n = 128	2.1 ± 0.4 a n = 81	17.8 ± 4.3 n = 135	7.2 ± 0.8 n = 84

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Table 4. Area- and seasonally-weighted annual estimates of N_2O, N_2, and total gaseous N flux

Elevation Band (m.a.s.l.)	Habitat	Surface Area (ha)	Fraction of Land Area	Fraction of Year Wet Season	Fraction of Year Dry Season	Nitrous Oxide Yield	Unweighted Nitrous Oxide Flux Wet Season kg N_2O-N ha^{-1} yr^{-1}	Unweighted Nitrous Oxide Flux Dry Season kg N_2O-N ha^{-1} yr^{-1}	Area-weighted Nitrous Oxide Flux Wet Season kg N_2O-N ha^{-1} yr^{-1}	Area-weighted Nitrous Oxide Flux Dry Season kg N_2O-N ha^{-1} yr^{-1}	Area-weighted and Seasonally-weighted Annual Estimate of N_2O Flux kg N_2O-N ha^{-1} yr^{-1}	Area-weighted and Seasonally-weighted Annual Estimate of N_2 Flux kg N_2-N ha^{-1} yr^{-1}	Area-weighted and Seasonally-weighted Annual Estimate of Total Gaseous N Flux kg N ha^{-1} yr^{-1}
600-1200	Premontane forest	733000	0.24	0.58	0.42	0.4 ± 0.05	2.59 ± 0.91	2.88 ± 0.95	0.63 ± 0.22	0.70 ± 0.23	0.66 ± 0.16	1.00 ± 0.29	1.66 ± 0.33
1200-2200	Lower montane forest	892000	0.30	0.58	0.42	0.19 ± 0.04	0.33 ± 0.29	3.72 ± 2.12	0.10 ± 0.09	1.10 ± 0.63	0.52 ± 0.27	2.21 ± 1.24	2.73 ± 1.26
2200-3200	Upper montane forest	807000	0.27	0.58	0.42	0.42 ± 0.05	0.22 ± 0.33	0.04 ± 0.40	0.06 ± 0.09	0.01 ± 0.11	0.04 ± 0.07	0.05 ± 0.09	0.09 ± 0.12
3200-3700	Montane grasslands	586000	0.19	0.58	0.42	0.61 ± 0.06	-0.04 ± 0.40	0.69 ± 0.44	-0.01 ± 0.08	0.13 ± 0.09	0.05 ± 0.06	0.03 ± 0.04	0.09 ± 0.07
Totals		3020000									**1.27 ± 0.33**	**3.29 ± 1.27**	**4.57 ± 1.31**

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Figure 1. Map of study sites across the Kosñipata Valley, Manu National Park, Peru.

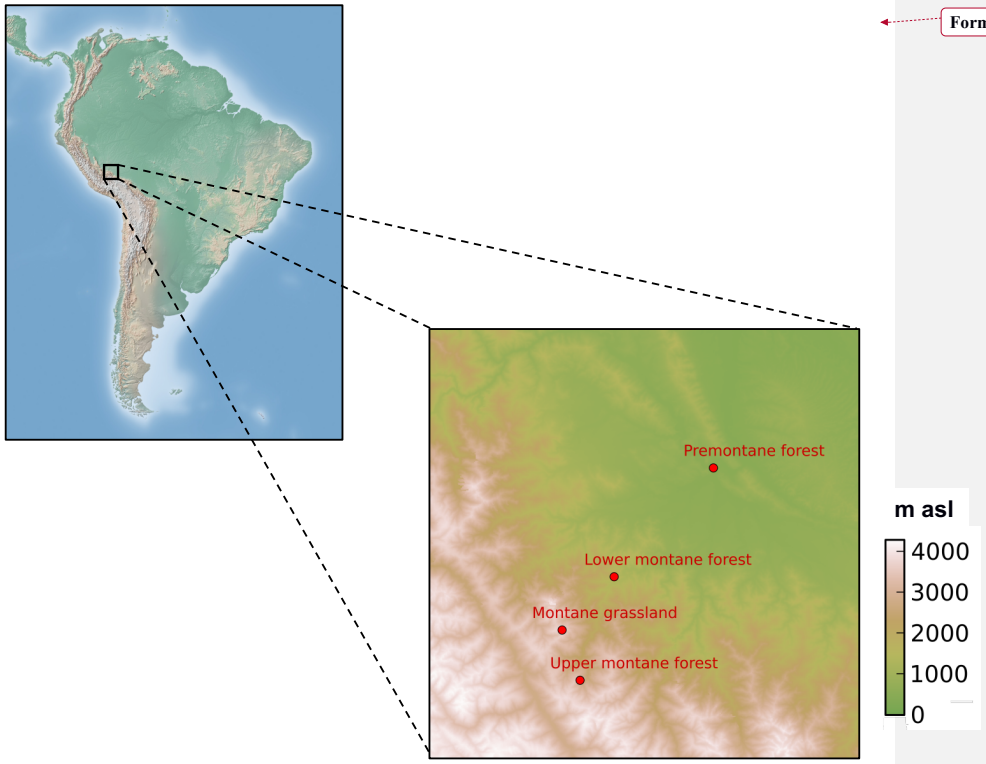

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Figure 2. Plot-averaged (a) net N_2O flux, (b) water-filled pore space, and (c) resin-extractable NO_3^- flux among habitats. Boxes enclose the interquartile range, whiskers indicate the 90th and 10th percentiles. Lower case letters indicate statistically significant differences among means (Fisher's LSD, $P < 0.05$).

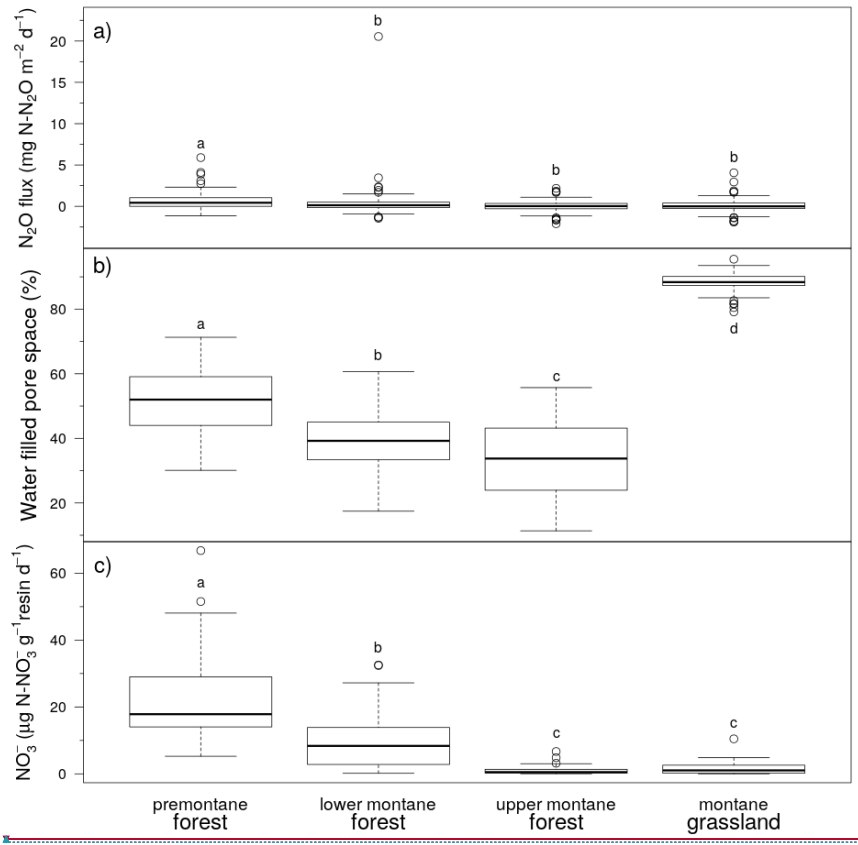

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Figure 3. Time series of net N_2O flux and water-filled pore space (WFPS). Panels indicate data for (a) premontane forest, (b) lower montane forest, (c) upper montane forest, and (d) montane grasslands for the 30-month study period beginning in January 2011 and ending in June 2013. The broken horizontal line running across each panel denotes the overall mean N_2O flux or WFPS for that habitat. The broken line in each box indicate median values and the black lines indicate means. Dry and wet seasons are denoted by vertical shading on the graph, with the dry season (May to September) highlighted in white and the wet season (October to April) in light blue.

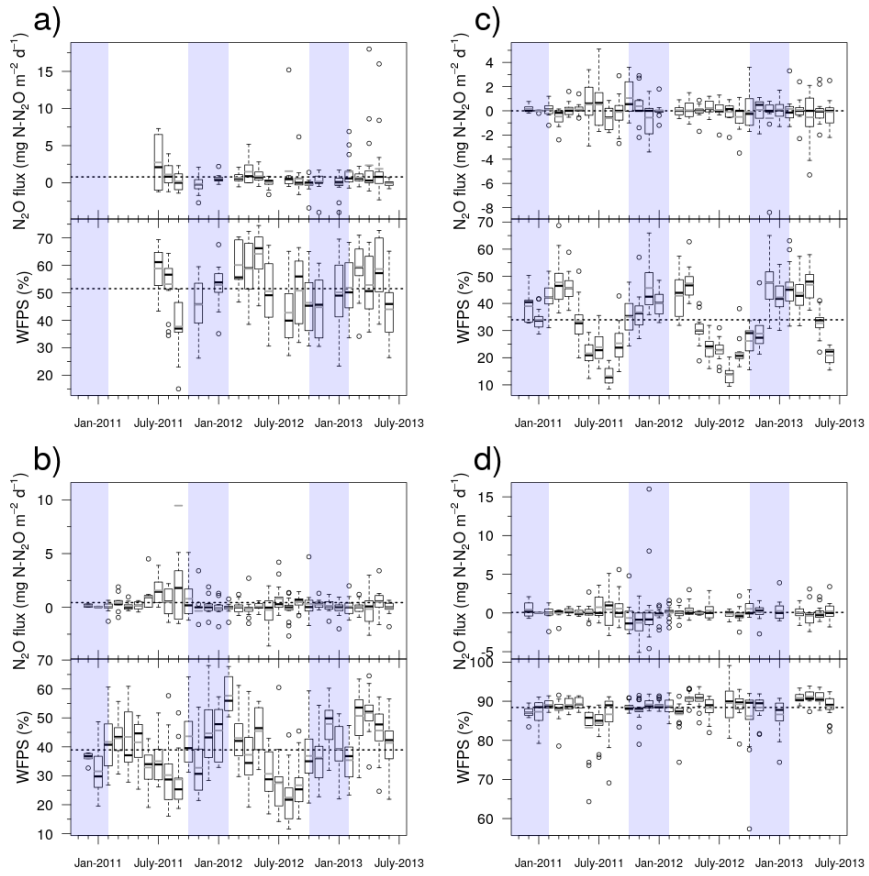

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Figure 4. Total (a) ^{15}N-N_2O flux and (b) ^{15}N-N_2 flux during the early (≤24 hours) and late (>24 hours) incubation phases of the water-filled pore space (WFPS) experiment. Results from the 90 % WFPS treatment are shown in dark-grey, while data from the 70 %, 50 %, and 30 % WFPS treatments are shown in mid-grey, light-grey, and white, respectively. The bar charts show means and standard errors.

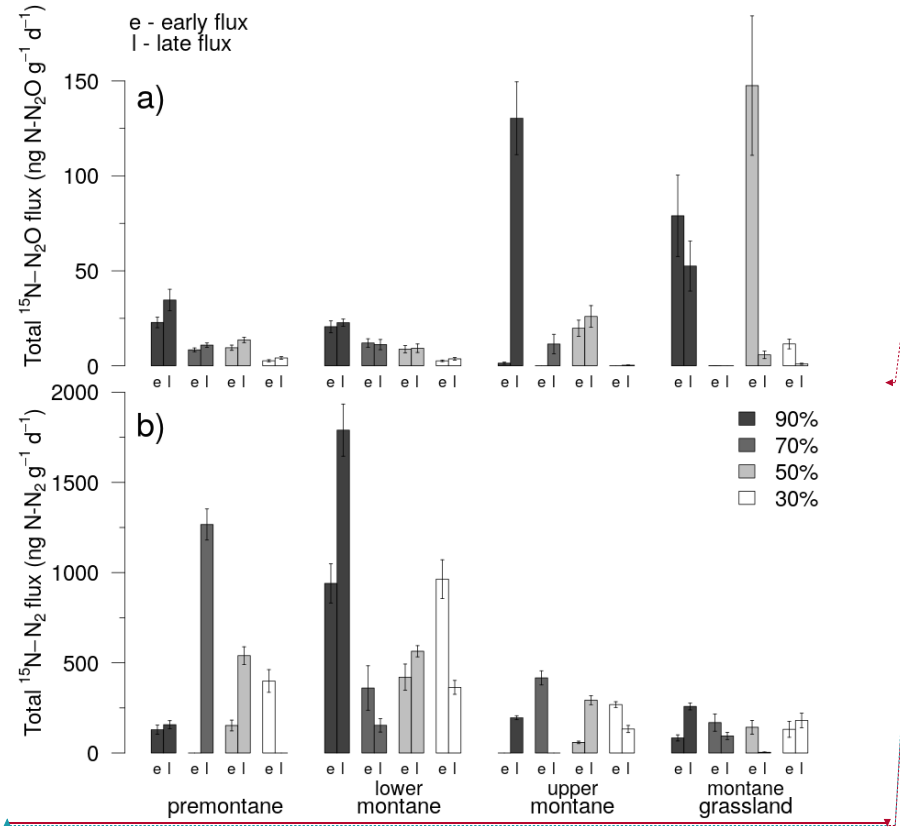

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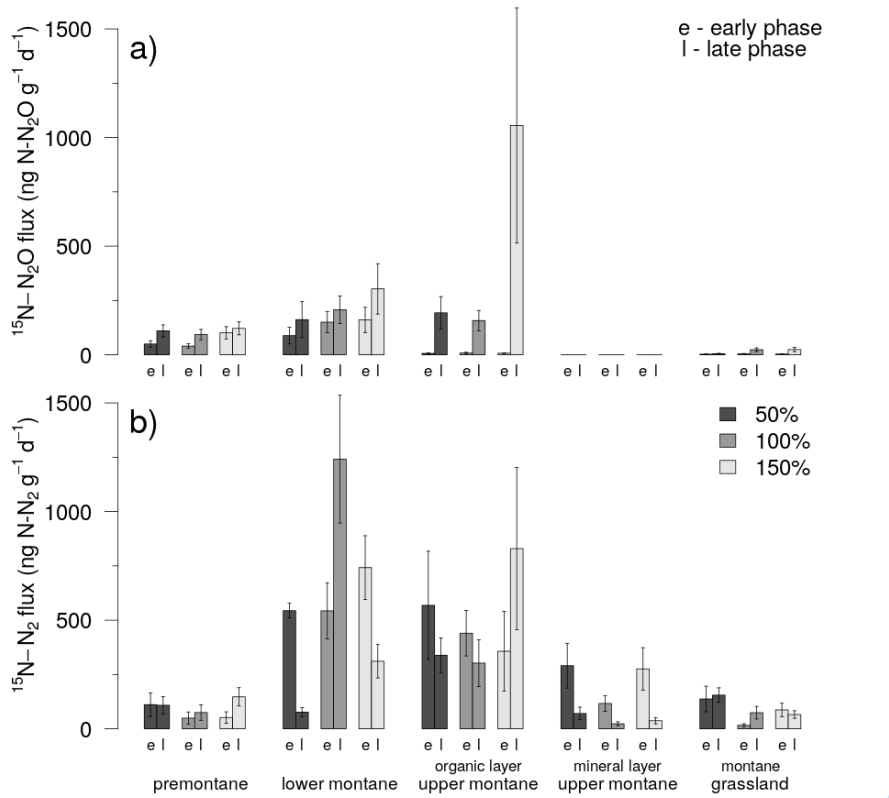
Figure 5. (a) ^{15}N-N_2O flux and (b) ^{15}N-N_2 flux during the early (≤24 hours) and late (>24 hours) incubation phases of the NO_3^- addition experiment. Results from the +50 % NO_3^- addition are shown in dark-grey, while data from the +100 % and +150 % treatments are shown in mid-grey and light-grey, respectively. The bar charts show means and standard errors.



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To address these hypotheses, we conducted a combined field and laboratory study, including monthly field flux measurements collected across a range of elevations and habitats over a 30-month period; a laboratory-based soil moisture manipulation experiment; a field-based litter-fall manipulation study; and a laboratory-based NO_3^- addition study.

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The habitat by incubation phase interaction indicated that some habitats showed different flux from each other during different phases of the incubation (Figure 4). For example, premontane and lower montane forest showed no significant difference in flux during different incubation phases (t-Test, $P > 0.05$ for each habitat), whereas upper montane forest mineral layer soils showed a significant increase from early to late incubation phases (5 ± 2 ng N_2O-^{15}N g^{-1} d^{-1} versus 42 ± 13 ng N_2O-^{15}N g^{-1} d^-1; t-Test, $P < 0.003$). In contrast to the other habitats, montane grasslands showed a significant decrease in flux from early to late incubation phases (60 ± 23 ng N_2O-^{15}N g^{-1} d^{-1} versus 6 ± 9 ng N_2O-^{15}N g^{-1} d^{-1}, respectively; t-Test, $P < 0.02$).

The habitat by moisture by incubation phase effect indicated that different habitats showed varying responses to moisture depending on the incubation phase (Figure 4). For example, for the premontane and lower montane forest, which showed no effect of incubation phase, flux followed the moisture trend described for the data set as a whole (i.e. highest flux for the 90 % WFPS treatment, lowest flux for the 30 % WFPS treatment, intermediate flux for the 50 & 70 % WFPS treatments). In contrast, for upper montane forest mineral layer soils, the effects of moisture varied with incubation phase. During the early phase, flux was highest in the 50 % WFPS treatment (20 ± 8 ng N_2O-^{15}N g^{-1} d^{-1}), while all other treatments showed lower flux (pooled average of 0.5 ± 0.4 ng N_2O-^{15}N g^{-1} d^{-1}). In the late phase, flux was highest for the 90 % WFPS treatment (145 ± 40 ng N_2O-^{15}N g^{-1} d^{-1}) while the other treatments were lower and not statistically different from each other (pooled average: 13 ± 5 ng N_2O-^{15}N g^{-1} d^{-1})

The habitat by incubation phase interaction indicates that flux for different habitats showed different patterns during early and late incubation phases (Figure 4). For example, premontane forest showed a significant increase for early (169 ± 42 ng N_2-^{15}N g^{-1} d^{-1}) to late (483 ± 91 ng N_2-^{15}N g^{-1} d^{-1}) incubation phases (t-Test, $P < 0.01$. In contrast, lower montane forest, upper montane forest mineral layer soil, and montane grassland all showed no significant change in flux between incubation phases (t-Test, $P > 0.05$ for all habitats).

Finally, the habitat by moisture level by incubation phase interaction indicates that moisture responses among habitats were influenced by incubation phase (Figure 4). For example, for the premontane forest, where an incubation phase effect was found, the response to moisture varied depending on incubation phase. During the early phase of the incubation, flux was lowest from the 70 % WFPS treatment (0 ± 0 ng N_2-^{15}N g^{-1} d^{-1}), while all other moisture treatments showed similar levels of flux (pooled average: 224 ± 52 ng N_2-^{15}N g^{-1} d^{-1}). For the late phase, the highest flux was observed for the 70 % WFPS treatment (1,267 ± 175 ng N_2-^{15}N g^{-1} d^{-1}), followed by the 50 % WFPS treatment (540 ± 99 ng N_2-^{15}N g^{-1} d^{-1}), the 90 % treatment (157 ± 43 ng N_2-^{15}N g^{-1} d^{-1}), and the 30 % WFPS treatment (0 ± 0 ng N_2-^{15}N g^{-1} d^{-1}) (Fisher's LSD, $P < 0.05$). In contrast, for all other habitats, where there was no significant incubation phase effect (i.e. lower montane forest, upper montane forest mineral layer soil, montane grassland), the response to moisture followed the overall pattern described previously.

For the upper montane forest mineral layer soil, N_2O yield shifted from 0.33 ± 0.07 to 0.51 ± 0.07 (t-Test, $P < 0.04$), while for montane grassland N_2O yield changed from 0.70 ± 0.07 to 0.52 ± 0.09 (t-Test, $P < 0.05$).

fact that the moisture response of different habitats was contingent upon incubation phase. For instance, for upper montane forest mineral layer soil, N_2O yield during the early phase was greatest for the 90 % WFPS treatment (1; i.e. no ^{15}N-N_2 flux observed), while the 50 % WFPS treatment showed intermediate N_2O yield (0.33 ± 12), and the 30 and 70 % WFPS treatments collectively showed the lowest N_2O yields (approximately 0 for both; i.e. no ^{15}N-N_2O flux observed) (Fisher's LSD, $P < 0.05$). In contrast, during the late phase, the 70 % WFPS treatment showed the highest N_2O yield (1; i.e. no ^{15}N-N_2 flux observed), while the other treatments showed lower N_2O yields that were not significantly different from each other (pooled average: 0.33 ± 0.07) (Fisher's LSD, $P < 0.05$). In contrast, for montane grassland, no effect of moisture was observed during the early phase of the incubation. However, during the late phase, the 50 % WFPS treatment showed the highest N_2O yield (0.89 ± 0.11), while the other treatments showed lower N_2O yields that were not significantly different from each other (pooled average: 0.39 ± 0.10) (Fisher's LSD, $P < 0.05$). For all other habitats with no habitat by phase interaction (i.e. premontane and lower montane forest), the moisture effect follows the general trends described above.

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further investigation revealed that this relationship arose from the fact that different habitats varied in their flux during early and late incubation phases (Figure 5). For example, during the early phase, lower montane and premontane forests collectively showed the highest flux (Figure 5; 133 ± 46 and 64 ± 19 ng N_2O-^{15}N g^{-1} d^{-1}, respectively) (Fisher's LSD, $P < 0.05$). Upper montane forest organic layer soils and montane grassland soils collectively showed intermediate rates of flux (Figure 5; 8 ± 2 and 4 ± 1 ng N_2O-^{15}N g^{-1} d^{-1}, respectively), while upper montane forest mineral layer soils showed the lowest flux (Figure 5; 0.04 ± 0.01 ng N_2O-^{15}N g^{-1} d^{-1}) (Fisher's LSD, $P < 0.05$). In contrast, during the late phase, upper montane forest organic layer soils, lower montane forest, and premontane forest now collectively showed the highest flux (469 ± 313 ng N_2O-^{15}N g^{-1} d^{-1}, 224 ± 85 ng N_2O-^{15}N g^{-1} d^{-1}, and 108 ± 25 ng N_2O-^{15}N g^{-1} d^{-1}, respectively). The lowest flux was from montane grasslands (18 ± 7 ng N_2O-^{15}N g^{-1} d^{-1}), followed by upper montane forest mineral layer soils (0.08 ± 0.02 ng N_2O-^{15}N g^{-1} d^{-1}) (Fisher's LSD, $P < 0.05$).

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6.1 Multi-annual trends in N_2O flux among habitats and between seasons

Montane forest and grassland ecosystems in the Kosñipata Valley were net sources of atmospheric N_2O, affirming our prior results (Teh et al., 2014). The flux for this multi-annual dataset were comparable to the preliminary values reported in our earlier publication, with mean flux of 0.27 ± 0.07 mg N-N_2O m^{-2} d^{-1} observed here over a 30 month period, compared with 0.22 ± 0.12 mg N-N_2O m^{-2} d^{-1} recorded over 13 months (Teh et al., 2014). Consistent with our earlier report, flux from our Peruvian transect were greater than those from a comparable study site in Ecuador (Wolf et al., 2011), which we attributed to higher N content in lower elevation soils in Peru (Teh et al., 2014). The elevational trends reported earlier still hold true for this multi-annual dataset (Teh et al., 2014); namely, significantly greater N_2O flux from lower elevation habitats (premontane forest, lower montane forest) compared to higher elevation ones (upper montane forest, montane grasslands) (Figure 2a). More favourable environmental conditions at lower elevations may explain these trends (e.g. higher N availability, warmer temperatures; see below for further details).

Nitrous oxide flux for the Kosñipata Valley varied between seasons, with significantly greater flux during the dry season compared to the wet season (Teh et al., 2014). However, this overall trend was strongly influenced by the behaviour of lower montane forest, which showed pronounced seasonality in N_2O flux, whereas the other habitats showed little or no seasonal differences (Table 3). For premontane forest, upper montane forest, and montane grassland, weak seasonality in N_2O flux may reflect the fact that environmental variables did not vary strongly between seasons (Table 3), challenging our first hypothesis (**H1**). Instead, environmental variables tended to vary more strongly among habitats (section 5.2). Analysis of the environmental data repeatedly demonstrated that habitat accounted for the largest proportion of variance in ANOVA models, with season accounting for a substantially smaller proportion of the variance or none at all. Moreover, in cases where environmental variables differed significantly between seasons, the actual numerical differences were often relatively slight (Table 3). For example, while WFPS varied significantly between seasons, the numerical difference in WFPS between dry season and wet season was 7.4 % WFPS for the pooled data; i.e. 52.1 ± 2.4 versus 59.5 ± 1.6 % WFPS, respectively. Likewise, oxygen in the 0-10 cm soil depth varied by less than 1 %, with a mean dry season value of 17.8 ± 0.3 % compared to a wet season value of 16.8 ± 0.4 %. Soil temperature varied by less than 1.2 °C, with a mean dry season value of 13.9 ± 0.4 °C compared to a wet season value of 15.1 ± 0.3

°C. Other variables, such as air temperature and resin-extractable NO_3^- did not vary significantly between seasons at all.

Lower montane forest is the only habitat that showed evidence of seasonal fluctuations in N_2O flux driven by variability in environmental conditions. This is evidenced by the results of multiple regression analysis of environmental variables against N_2O flux (section 5.3). Key variables found to influence N_2O flux included air temperature, soil temperature, WFPS, and resin-extractable NH_4^+ flux. According to the multiple regression analysis, the dominant environmental regulator for N_2O flux was air temperature, which showed a negative relationship with N_2O flux. While we are not entirely certain why air temperature was negatively correlated with flux; one possible explanation is that this relationship reflects the effect of air temperature on some other process linked to N_2O flux, such as drying of surface soil layers. Higher air temperatures may have led to increased evaporation in surface soil horizons, reducing rates of N cycling. This is a phenomenon we have observed in other warm, seasonally-dry environments (Teh et al., 2011), and we found limited evidence for this interpretation of the data in the weak but statistically significant inverse relationship between air temperature and WFPS (r^2 = 0.12, P < 0.002; data not shown). The positive relationship between soil temperature is perhaps more intuitive to interpret, and may reflect enhanced microbial activity as the soil warms. Likewise, the negative relationship with WFPS and N_2O flux probably reflects enhanced N_2O reductase activity and greater denitrification to N_2 with increasingly anaerobic conditions (Morley and Baggs, 2010;Morley et al., 2008). Last, the inverse relationship between resin-extractable NH_4^+ and N_2O flux may reflect competition for NO_3^- between denitrification and dissimilatory nitrate reduction to ammonium (DNRA), the two nitrate-reducing processes that are believed to be relatively common in wet, organic matter-rich tropical soils (Silver et al., 2001). Of course, one puzzling feature of this data is the divergent relationships that air temperature and soil temperature show with N_2O flux. We believe that the most likely explanation for this is that these two environmental variables are, to some extent, decoupled from each other in these montane habitats, leading to the two variables behaving differently from each other and acting as least quasi-independently on N_2O flux. This is evidenced by the weak positive correlation between air and soil temperature in lower montane forest (r^2 = 0.20, P < 0.0001), which suggests that a large proportion of the variance in soil temperatures (i.e. up

to 80 %) are explained by other environmental factors, and not by ambient air temperature alone. However, it is important to note that interpretation of these results must be treated with some caution, given that the model as a whole was only on the borderline of statistical significance ($P < 0.07$, $r^2 = 0.36$).

One other important difference between this publication and our earlier work is that topography no longer appears to be an important driving variable in this multi-annual dataset. While the basin landform showed significantly lower N_2O flux than the other landforms when the effect of topography was investigated in isolation, a more comprehensive statistical analysis, which included topography and other variables (e.g. habitat, season, environmental conditions), suggests that topography is not a significant predictor of N_2O flux. Instead, the effects of topography may be contingent upon or co-vary with habitat, rather than acting independently of it.

6.2 Effects of soil moisture on N_2O flux

Results from our laboratory-based WFPS manipulations suggest that soil moisture content plays a significant role in modulating N_2O flux. This finding is noteworthy because our prior research suggested that there was no direct relationship between N_2O flux and WFPS (Teh et al., 2014), and challenged our broader theoretical understanding of the role that soil moisture plays in regulating N_2O flux (Firestone and Davidson, 1989;Firestone et al., 1980;Weier et al., 1993). However, the response of $^{15}N\text{-}N_2O$ flux and other response variables (e.g. $^{15}N\text{-}N_2$ flux, N_2O yield) were complex and non-linear, falsifying our second hypothesis (**H2**). Rather than $^{15}N\text{-}N_2O$ flux increasing progressively with WFPS, as predicted by **H2** and denitrification theory (Firestone and Davidson, 1989;Firestone et al., 1980;Weier et al., 1993), we observed two distinct and separate peaks in $^{15}N\text{-}N_2O$ flux. The highest $^{15}N\text{-}N_2O$ flux was observed in the 90 and 50 % WFPS treatments, while the 30 and 70 % WFPS treatments showed significantly lower flux (Fisher's LSD, $P < 0.05$; Figure 4). This unexpected result may reflect competition for substrates (e.g. NO_3^-, labile organic C) among nitrate-reducing processes such as denitrification and DNRA (Silver et al., 2001), or may indicate that N_2O is being produced from DNRA (Streminska et al., 2012).

^{15}N-N_2 flux and N_2O yield also showed intriguing and unexpected trends. For example, ^{15}N-N_2 flux was highest flux in the 90 % WFPS treatment (Fisher's LSD, P < 005), but did not differ significantly among the other treatments (Figure 4). Likewise, N_2O yield was highest in the 70 % WFPS treatment (0.51 ± 0.06), above and below which significantly smaller proportions of ^{15}N were emitted as N_2O (Fisher's LSD, $P < 0.05$). These results are surprising because denitrification theory predicts that decreases in WFPS should lead to a reduction in N_2 flux and increases in N_2O yield (Firestone and Davidson, 1989;Firestone et al., 1980;Weier et al., 1993), as N_2O reductase is increasingly suppressed by drier and more oxic soil conditions (Burgin and Groffman, 2012;Weier et al., 1993;Firestone et al., 1980;Morley and Baggs, 2010;Morley et al., 2008). One explanation for this is that N_2O production under drier conditions (i.e. <50 % WFPS) may be occurring in anaerobic microsites (Keller et al., 1993;Silver et al., 1999).

6.3 N_2O flux not constrained by labile organic matter availability

Nitrous oxide flux was unaffected by variations in leaf litter-fall, partially challenging our third hypothesis (**H3**). This finding runs counter to the results from lowland tropical forests (Sayer et al., 2011), where trace gas flux can be strongly influenced by changes in labile organic matter inputs, such as leaf litter. The relative insensitivity of these montane ecosystems to changes in leaf litter-fall, a proxy for labile organic matter inputs, may be due to the relatively large size of soil organic matter pools in these soils (Zimmermann et al., 2012, Zimmermann et al., 2009a, Zimmermann et al., 2010b), which could buffer N_2O production against short-term fluctuations in labile organic matter availability. Moreover, because of the relatively large soil organic matter stocks, and N_2O emission could be more strongly constrained by other factors, such as N availability, soil WFPS or pH. This finding is significant for understanding and modelling process-based controls on N_2O flux, as many bottom-up, process-based models assume that N cycling and turnover of labile organic matter are linked through processes such as litter production and decomposition (Li et al., 2000;Werner et al., 2007). While not disproving these assumptions, these data suggest that the linkage between litter production and N_2O flux are weak in these montane environments.

6.4 Importance of NO_3^- in regulating N_2O flux

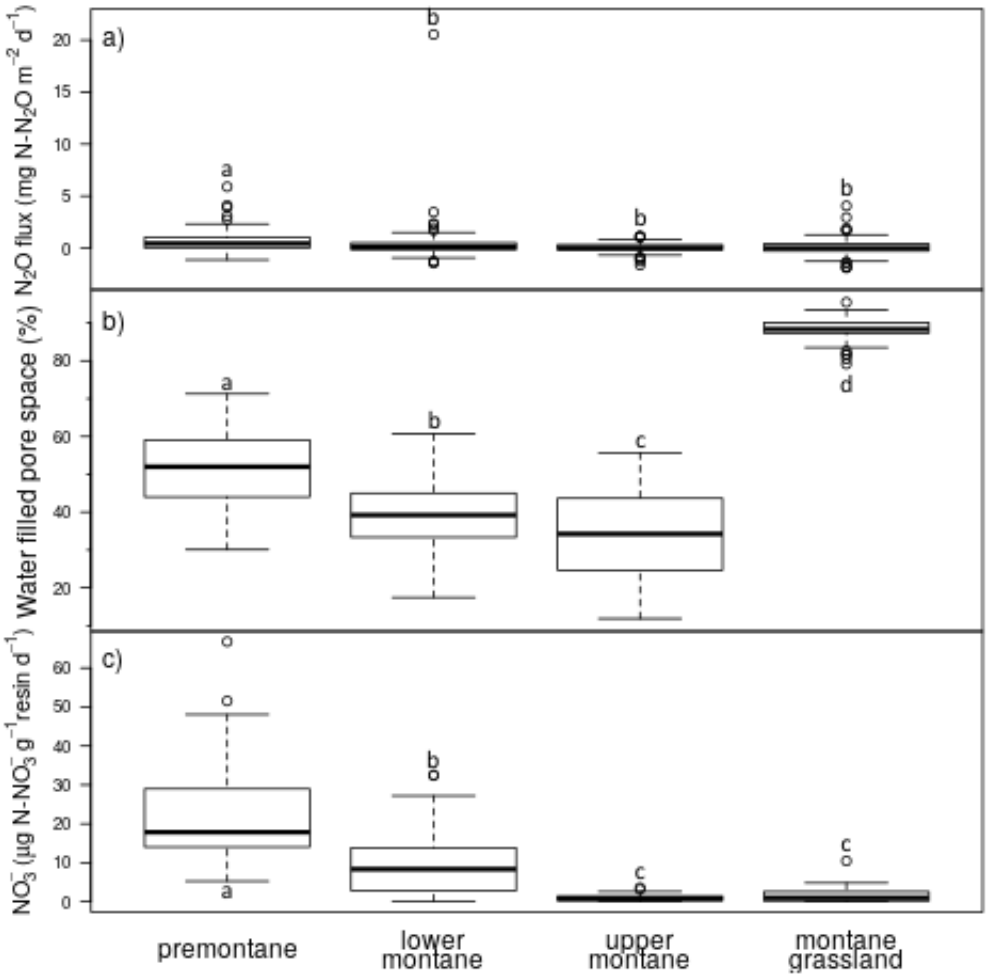
One of the principal hypotheses raised by our earlier research is that N_2O flux is strongly limited by NO_3^- across this tropical elevation gradient (Teh et al., 2014). The detailed, process-oriented studies conducted here provide evidence that supports this claim, indicating that longer-term, time-averaged patterns in NO_3^- availability among habitats influence N_2O flux. The strongest evidence comes from the ^{15}N-N_2O flux data from our ^{15}N-NO_3^- addition experiment. Trends in ^{15}N-N_2O flux echoed patterns in our field data and prior denitrification potential experiments (Teh et al., 2014). Namely, we observed an inverse trend in ^{15}N-N_2O flux with elevation, with significantly higher ^{15}N-N_2O flux from lower elevation premontane (86 ± 16 ng N_2O-^{15}N g^{-1} d^{-1}) and lower montane (179 ± 48 ng N_2O-^{15}N g^{-1} d-1) forests, compared to higher elevation upper montane forest mineral layer soils (0.06 ± 0.01 ng N_2O-^{15}N g^{-1} d^{-1}) and montane grasslands (11 ± 4 ng N_2O-^{15}N g^{-1} d^{-1}) (Figure 5a). This pattern in ^{15}N-N_2O flux follows trends in resin-extractable NO_3^- flux, implying that NO_3^- may constrain the potential of these soil to emit N_2O (Figure 2a-b, Figure 5a) (Teh et al., 2014). The exception to this pattern is upper montane forest organic layer soils, which showed the highest flux when incubated under laboratory conditions (Figure 5). However, it is important to note that the significantly lower bulk density of the organic horizon in upper montane forests (~0.06 g cm^{-3} for the O horizon versus ~0.6 g cm^{-3} for the mineral horizon) means that this O layer makes a smaller proportional contribution to N_2O flux than soils from lower mineral horizons (Zimmermann et al., 2009a;Zimmermann et al., 2009b).

Furthermore, the behaviour of the NO_3^- amended soils during the early (≤24 hours) and late (>24 hours) phases of the incubation suggest that soils from more N-poor habitats showed a greater proportional increase in ^{15}N-N_2O flux following NO_3^- addition than N-rich habitats, suggesting that ^{15}N-N_2O flux was more NO_3^- limited in N-poor environments (Figure 5). For example, soils from the upper montane forest organic layer, montane grasslands, and upper montane forest mineral layer showed the lowest early phase ^{15}N-N_2O flux, but the greatest proportional increase in flux during the late incubation phase, rising by a factor of 59, five, and two, respectively. In contrast, lower montane and premontane forest soils, which showed the highest NO_3^- availability and N_2O flux in the field, and the greatest early phase ^{15}N-N_2O flux in the incubations, showed the smallest proportional increase in the late incubation phase (i.e. 1.7 times increase). Overall, these data imply that ^{15}N-N_2O flux from

N-poor habitats are more strongly NO_3^- limited, whereas N_2O flux from more N-rich soils may be more heavily constrained by other environmental factors.

The other field and laboratory data were more equivocal, reflecting the complex and potentially confounding environmental controls on N_2O flux (Groffman et al., 2009). For example, while lower N_2O flux was associated with more N-poor habitats, N_2O flux was only weakly correlated with resin-extractable NO_3^- flux (r^2 = 0.03, P <0.03). Moreover, for the laboratory-based NO_3^- addition experiment, we found no evidence that these soils responded to short-term increases in NO_3^- availability, at least within the concentration range that we used in this experiment. $^{15}N\text{-}N_2O$ flux, $^{15}N\text{-}N_2$ flux, and N_2O yield were not directly influenced by the amount of $^{15}N\text{-}NO_3^-$ added (Figure 5). Rather, ANCOVA suggests that $^{15}N\text{-}N_2O$ and $^{15}N\text{-}N_2$ fluxes were better-predicted by habitat. N_2O yield, normally a sensitive indicator of NO_3^- availability (Blackmer and Bremner, 1978;Weier et al., 1993;Parton et al., 1996), showed no immediate response to the amount of $^{15}N\text{-}NO_3^-$ added, nor any of the other explanatory variables. One explanation for this, consistent with the notion that N_2O flux is NO_3^- limited, is that nitrate-reducing microbes in these soils may have a relatively low half-saturation constant (K_m) for NO_3^-, and effectively utilize NO_3^- whenever concentrations increase above background levels (Holtan-Hartwig et al., 2000). As a consequence, we may be unable to differentiate among NO_3^- treatments because the NO_3^- addition levels that we used all exceeded the K_m for in these soils. This finding is also consistent with results from long-term N fertilization studies, which suggest that substantive shifts in N_2O flux are only likely to occur after prolonged exposure to high levels of N, rather than due to transient fluctuations in N availability (Hall & Matson 1993; Koehler et al 2009; Corre et al 2014).

Habitat	N_2O mg N-N_2O m^{-2} d^{-1}		WFPS %		Soil Temperature °C		Air Temperature °C		Oxygen %		NO_3^- µg N-NO_3^- (g resin)$^{-1}$ d^{-1}		NH_4+ µg N-NH_4^+ (g resin)$^{-1}$ d^{-1}	
	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season
Premontane	0.71 ± 0.25 a n = 130	0.79 ± 0.26 a n = 98	51.9 ± 1.6 a n = 135	51.2 ± 2.1 a n = 135	20.7 ± 0.1 a n = 143	20.2 ± 0.1 b n = 120	21.5 ± 0.3 n = 143	20.4 ± 0.5 n = 120	19.4 ± 0.2 a n = 52	19.6 ± 0.2 a n = 36	23.2 ± 3.6 a n = 89	22.1 ± 2.1 a n = 96	31.4 ± 13.0 n = 90	11.3 ± 1.8 n = 95
Lower montane	0.09 ± 0.08 a n = 212	1.02 ± 0.58 b n = 137	42.2 ± 1.0 a n = 271	34.0 ± 1.4 b n = 179	18.1 ± 0.1 a n = 254	17.3 ± 0.2 b n = 164	18.9 ± 0.3 n = 254	18.3 ± 0.2 n = 164	19.2 ± 0.2 a n = 146	19.2 ± 0.1 a n = 81	11.8 ± 1.9 a n = 123	7.8 ± 1.4 a n = 94	20.2 ± 5.4 n = 124	8.6 ± 0.9 n = 93
Upper montane	0.06 ± 0.09 a n = 207	0.01 ± 0.11 a n = 146	42.0 ± 1.3 a n = 264	24.3 ± 1.4 b n = 180	11.8 ± 0.1 a n = 255	10.9 ± 0.2 b n = 165	12.8 ± 0.2 n = 255	12.5 ± 0.3 n = 165	18.7 ± 0.2 a n = 165	18.5 ± 0.2 a n = 109	1.4 ± 0.2 a n = 128	0.6 ± 0.2 b n = 91	22.5 ± 6.3 n = 129	11.3 ± 1.4 n = 93
Montane grassland	-0.01 ± 0.11 a n = 238	0.19 ± 0.12 a n = 160	88.5 ± 0.3 a n = 303	88.3 ± 0.5 a n = 184	11.6 ± 0.1 a n = 282	9.0 ± 0.2 b n = 205	11.4 ± 0.3 n = 284	12.0 ± 0.5 n = 205	12.2 ± 0.9 a n = 176	15.4 ± 0.8 b n = 117	1.5 ± 0.4 a n = 128	2.1 ± 0.4 a n = 81	17.8 ± 4.3 n = 135	7.2 ± 0.8 n = 84



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