

***Interactive comment on* “The effect of flooding on the exchange of the volatile C<sub>2</sub>-compounds ethanol, acetaldehyde and acetic acid between leaves of Amazonian floodplain tree species and the atmosphere” by S. Rottenberger et al.**

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This submission examines the effect of root flooding on emissions of ethanol, acetaldehyde and acetic acid from the leaves of four tree species occurring in seasonally flooded Amazon forests. Several previous studies, carried out on temperate tree species, have clearly demonstrated that root hypoxia brought on by flooding can result in greatly enhanced emissions of these C-2 compounds, particularly acetaldehyde. Based on these studies, it has long been assumed that seasonally flooded tropical forests represent a potentially large source of these compounds to the atmosphere

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during at least part of the year. This manuscript represents the first attempt to examine this assumption; although ultimately it will be necessary to conduct above-canopy flux measurements in situ, these studies using potted Amazonian plant species represent a reasonable and important starting point.

Concerning the major thrust of the paper, I find little to criticize. All four floodplain species examined exhibited enhanced emissions of ethanol (apparently underestimated by as much as a factor 10 due to fragmentation within the PTR-MS drift tube) and acetaldehyde, although there was considerable variation between species in magnitude of emissions, duration of emissions and compositional ratios of emissions. The authors attempt to explain this variation on the basis of previously studied differences in their root morphologies and root physiology. This is most successful in the case of *Salix martiana*, which exhibited the lowest emissions of both ethanol and acetaldehyde, and in contrast to the other species exhibited only a very small early morning peak in emissions. Furthermore, its leaf level physiology (net photosynthesis and transpiration) seemed little affected by flooding. Only *S. martiana* developed adventitious roots in the days following flooding, and a previous study had indicated that the root cortex of *S. martiana* has large air spaces and that there is no significant induction of alcohol dehydrogenase (ADH) activity in flooded roots. Although neither root and stem ethanol concentrations nor ADH activities were measured in this study, there is considerable circumstantial evidence to suggest that aerobic respiration continues in the roots of *S. martiana*, with very limited ethanolic fermentation. Thus, this species has the ability to avoid the hypoxia-inducing effects of flooding, maintain more or less normal physiology. With little transport of ethanol from roots to leaves, there is little emission of ethanol and its oxidation product, acetaldehyde, from the leaves to the atmosphere.

On the other extreme, *Laetia corymbulosa* exhibited by far the highest acetaldehyde emissions as well as high ethanol emissions following flooding, consistent with a lack of root air spaces and the previously reported 10-fold increase in ADH activity. Additionally, it was the only species examined in which (small) emissions of acetic acid

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(produced from acetaldehyde by the action of aldehyde dehydrogenase (ALDH)) were observed. Increased ethanolic fermentation in the roots, transport to the leaves and subsequent metabolism there clearly explains the observed increase in emissions. The authors link this inability to avoid root hypoxia with "strong reduction in (leaf) physiological activities." I was struck however by the ability of these leaves to maintain rates of photosynthesis and transpiration over 50% of their pre-flooding levels. Despite the energetic shortcomings of ethanolic fermentation, these plants are also able to adapt to hypoxia and maintain significant metabolic activity in the shoots, although there is clearly a metabolic cost involved.

Rather than dwell on the two species exhibiting somewhat intermediate behavior, suffice it to say that neither avoids a certain level of root hypoxia, as evidenced by significant leaf emissions of ethanol and acetaldehyde, but that each appears to maintain shoot level metabolism (even enhanced over the first several days in *Tabernaemontana*) through some combination of root morphological adaptations and enhanced levels of fermentation.

Although I found the correlations between leaf emissions and root morphology/physiology compelling, I was less convinced by the authors attempts to invoke differences in leaf enzyme activity in explaining differences in either the magnitude of emissions or the ratio of ethanol:acetaldehyde:acetic acid. Even if 95% of the ethanol transported to the leaf is re-captured via ADH, the variable fraction leaking from the leaf would significantly upset the ethanol:acetaldehyde emission ratio, independent of enzyme activities. Furthermore, the ethanol emission estimates themselves are highly uncertain, due to the fragmentation of mass 47 in the PTR-MS. Emissions of acetic acid, seen only in *L. corymbulosa*, which also exhibited by far the highest acetaldehyde emissions, seems to me more likely to result from the inability of acetyl-CoA synthetase to rapidly activate all the acetic acid produced to acetyl-CoA than to increased ALDH activity (which is required in all the species to efficiently metabolize the highly toxic acetaldehyde). So although I agree with the authors than the regulation of these

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emissions is highly complex, depending on ethanol production in the roots, transport to the leaves (to some extent at least dependent on stomatal conductance), and enzymatic activity in the leaves themselves, I think it's premature to ascribe variations in emissions to significant variation in the activities of ADH and ALDH, particularly since they weren't measured.

Finding little to criticize in the methodology or general conclusions of the manuscript, I'd like to call attention to a relatively minor point, namely the apparent difficulty of reconciling measured deposition velocities of acetaldehyde and measured/calculated values of stomatal conductance (in non-flooded plants). The authors conclude, as they have in previous studies (Rottenberger et al., Ecol. App. 14 (Supplement):S247-S262, 2004), that there must be substantial deposition to the leaf cuticle. The discrepancy between measured deposition velocity and stomatal conductance to acetaldehyde, indicating a significant non-stomatal deposition, represents only a very minor part of this manuscript. However, it runs counter to my (quite limited) experience, in which acetaldehyde uptake can be explained largely by uptake through the stomata. Certainly, the suggestion that less than 25% of the uptake in *P. glomerata* can be explained by stomatal conductance (stomatal conductance to acetaldehyde =  $0.05 \text{ cm s}^{-1}$  while measured deposition velocity was  $0.24 \text{ cm s}^{-1}$ ) requires some explanation. What value for the ratio of diffusivities ( $\text{H}_2\text{O}$  vs. acetaldehyde) was used to calculate conductance to acetaldehyde, and what is the uncertainty associated with this value? How confident are the authors in their stomatal conductance measurements? For example, were values of calculated internal  $\text{CO}_2$  ( $C_i$ ) within a reasonable range (240 ppm +/- 40)? Does deposition continue when stomata are closed at night (if they fully close)? And finally, what might be the mechanism for non-stomatal deposition? Within the leaf, acetaldehyde is presumably being rapidly metabolized by either the ADH or ALDH reactions, maintaining the concentration gradient between the atmosphere and the leaf internal air space. But what sustains the gradient if acetaldehyde is simply sticking to the cuticle? Or is it somehow being metabolized, perhaps by leaf microbes?

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These minor points aside, simply characterizing the emissions of C-2 compounds from these four species of Amazon floodplain species, and relating the observations to root morphology and physiology justifies publication of this work. Obviously, and as the authors are well aware, it represents only a small first step towards characterizing the impact of such emissions from seasonally flooded tropical forests at the ecosystem or regional scales. How well do flooded pots mimic the root environments of trees in the field? Is avoidance of root hypoxia, as seen in *S. martiana*, a common adaptation to flooding in the tropics, or is some level of hypoxia with subsequent induction of ADH and ethanolic fermentation more typical? The data presented here suggest that after the first few days of greatly enhanced emission activity, emissions subsequently drop to quite low levels again. Only longer term studies, preferably in situ, will reveal whether enhanced emissions in the field are characterized by a short-term burst or a sustained flux to the atmosphere. And finally, of course, above canopy flux measurements, now feasible with PTR-MS eddy covariance, will be necessary to fully determine the impact at regional scales.

A few more or less significant comments follow:

p. 464, l. 8 no significant emissions? What is significant? Any emissions are certainly quite small compared to post-flooding emissions, but if all trees emitted at these "insignificant" rates, it would represent a significant global flux.

p. 464, l. 21 although I don't seriously question the suggestion that these C-2 emissions are arising from ethanol transported from roots to leaves, I don't think one can use the temporal correlations of emissions as proof; after all, each of these reactions is reversible, and the temporal correlation would also exist if acetaldehyde were being produced from pyruvate in the leaves (PDC) and then going on to produce ethanol (ADH), as apparently occurs during the so-called PDH bypass.

p. 465, l. 6 although the toxicity of acetaldehyde is clear, I seem to recall reading that the high toxicity of ethanol has been questioned recently

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p. 468, l. 5 I suspect "18-36C" should be "28-36C"

p. 469, l. 12 Although I'm no expert in PTR-MS techniques, it is not clear to me why a signal at  $m/z$  29 is "obviously not detectable". Are there any known interferences with the measurement of acetic acid at  $m/z$  61? Please make clear whether the concentrations/emissions of ethanol are based solely on  $m/z$  47 or whether any correction was applied.

p. 469, l. 8 This sentence "For each cuvette . . ." is unclear to me.

p. 470, l. 7 How were HPLC measurements calibrated?

p. 470, l. 19 Sentence "For acetic acid . . ." is unclear to me.'

p. 472, l. 19 How were Oxygen concentrations measured?

p. 473, l. 13 There seems to be some discrepancy between the PTR-MS data (Fig. 2) and the HPLC data (Fig. 3). While the PTR-MS data for *P. glomerata* suggests almost zero acetaldehyde exchange, the DNPH data indicate that emission and deposition rates could reach values as high as  $9 \text{ nmol m}^{-2} \text{ min}^{-1}$  when ambient acetaldehyde concentrations were very low or greater than 2.5 ppb. Is this just a function of the PTR-MS detection limit? How much did ambient acetaldehyde concentrations vary during the day, and why did it vary?

p. 474, l. 27 stomata closed, but not completely (Fig. 2)

p. 478, l. 24 I don't understand how the increased solubility of acetic acid might explain the different deposition behavior of acetic acid and acetaldehyde. Higher solubility might decrease the mesophyll resistance term, but since acetaldehyde deposition already greatly exceeds stomatal conductance,  $R_m$  appears negligible to begin with. Why might acetaldehyde be deposited to the cuticle, as suggested by the authors, but not acetic acid?

p. 479, l. 28 I'm not sure the important work of Niinemets et al. is relevant here. High

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solubility only leads to short-term stomatal control; at steady-state, air-space concentrations should increase sufficiently to overcome changes in stomatal conductance.

p. 481, l. 8 On the basis of Fig. 4, the highest ethanol emissions arise from *T. juruana* rather than *L. corymbulosa*

p. 485, l. 1 If the data are available, I'd be curious to know how isoprene emissions in *L. corymbulosa* responded to the flooding conditions imposed in this study.

A number of very minor grammatical, spelling, syntax suggestions follow:

p. 465, l. 15 "Inundation poses. . ."

p. 465, l. 20 not all "European"; suggest "temperate tree species"

p. 467, l. 12 "representative of . . ."

p. 467, l. 14 & 19 suggest deleting "stem"

p. 467, l. 20 Suggest "In nature, all species experience similar water regimes."

p. 467, l. 21 "2-3 year old"

p. 467, l. 24 "watered daily"

p. 472, l. 22 "hypoxic rather than anoxic. . ."

p. 473, l. 5 insert comma after "VOCs"

p. 474, l. 7 Suggest "While" for "Whilst"

p. 474, l. 10 suggest "least pronounced"

p. 474, l. 20 "acetaldehyde emissions"

p. 474, l. 22 ". . . different than that of other species"

p. 475, l. 3 "in none of the species was diurnal emission variability directly . . ."

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- p. 475, l. 7 Panels in Fig. 4 are not labelled "a" and "b"
- p. 475, l. 15 suggest "consistently low"
- p. 476, l. 5 ". . . 24 days, both emissions . . ."
- p. 476, l. 9 "and" for "und"
- p. 476, l. 24 Suggest "Although in principal, ethanol recovery . . . should lead to . . ."
- p. 476, l. 27 X-axis unlabelled in Fig. 5
- p. 478, l. 12 "range of 0.1 to 1.1 ppb"
- p. 479, l. 4 "for the first time that flooding. . ."
- p. 479, l. 13 "where it can be metabolized"
- p. 480, l. 20 suggest deleting "potential"
- p. 482, l. 9 "similar to those of *L. corymbulosa*"
- p. 482, l. 18 Don't understand sentence beginning "The adaptation mechanisms. . ."
- p. 482, l. 23 "In the case of *T. juruana*. . ."
- p. 482, l. 27 suggest "overlooked" for "overseen"
- p. 483, l. 9 suggest "ratio" instead of "mixture"
- p. 484, l. 13 ". . . transpiration were higher for this species. . ."
- p. 486, l. 13 suggest "other observations on each species' anatomy . . ."
- p. 496, Fig. 4 Sections A and B not indicated on Figure. Text in Panel A is quite small.

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