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## ***Interactive comment on “Stable carbon isotope fractionation during methanogenesis in three boreal peatland ecosystems” by P. E. Galand et al.***

### **Anonymous Referee #1**

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The paper by Galand et al. strengthens the notion that oligotrophic wetlands exhibit decomposition paths that differ significantly from their more minerotrophic counterparts. Despite the fact, that some studies have demonstrated active acetoclastic methanogenesis and the presence of acetate-consuming methanogens in relatively oligotrophic sites, studies continue to appear that show the dominance of hydrogenotrophic methanogenesis in oligotrophic wetlands. This study is a nice example in which oligotrophic sites were compared to a mineral-rich fen and only the latter supported significant acetotrophy. Hence, it is becoming increasingly clear that this phenomenon is widespread. The three sites studied were part of previous investigations showing the differences in composition of the methanogen populations that agree with the incubation and isotopic data presented in the current paper. The conclusion that the path to terminal products differs greatly between the minerotrophic fen and the two olig-

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otrophic sites, the latter being an incomplete degradation, is supported by the isotopic data, and the authors present a hypothetical degradation pathway that might explain how these differences occur. Factors controlling the methane/carbon dioxide ratio in wetlands need to be elucidated and this paper adds a piece to that quest. Previous studies have suggested that humic materials might act as electron sinks in wetlands leading to high methane/carbon dioxide ratios. The incomplete pathway presented in the current paper offers a somewhat similar scenario in which organic intermediates serve as oxidants, thus leading to excess carbon dioxide production relative to methane. In my opinion, the incomplete path depicted may also be equated to fermentation pathways that do not result in complete degradation. Regardless of the path, this work clearly underscores important differences in carbon flow, processes that need to be worked out to fully understand the impact of a warming world on gas exchange from northern wetlands.

The study used a combination of incubation and isotopic data for comparing C flow among the different sites. The isotopic data are the most compelling, but when combined with the methyl fluoride treatments, it becomes clearer that acetoclastic methanogenesis is inhibited to some degree in the nutrient-poor sites. Hence, the data provide strong evidence to support these differences.

Previous studies (cited in this paper) using isotopic methods and temporal changes in gas and solutes in incubations as well as isotopic data from field samples, yielded similar conclusions to these, but there are some interesting differences. The current study found highest rates of acetate accumulation and methane and carbon dioxide production in the minerotrophic MES site with much lower rates at the two nutrient-poor sites. Others found that acetate accumulated to very high levels in the most oligotrophic peats, whereas carbon dioxide and methane production was highest in minerotrophic sites. It has also been reported that acetate accumulated significantly in some minerotrophic sites, but completely turned over in others. The Galand et al. acetate data are final concentrations only so there is no way of knowing what the

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initial concentrations were, but differences in concentrations due to the methyl fluoride inhibition make it clear that acetate was indeed readily produced. Although both data from some other studies are reported using different units (volume vs. dry weight), it is still possible to compare rates.

The rates of production of gaseous products at the MES site in the current study are high at nearly 400 nmol g<sup>-1</sup> h<sup>-1</sup> for gases alone. Rates at the nutrient-poor sites are 5-10 times lower. These methane production rates at MES are ~13-times higher than those reported by Juottonen et al. (2005) for this same site, whereas rates for sites OLI and OMB do not differ greatly from those reported by Juottonen et al. (2005). Hence, samples from MES in the current study seem quite unusual in that rates are quite high and acetate accumulated to very high levels, even without inhibition by methyl fluoride. Since the MES data are the baseline by which the two oligotrophic sites are compared, it would be useful if the authors addressed what might be the cause of these discrepancies. I do not believe this affects the conclusion that methane sources differ between this site and the two others. The paper is focused very strongly on that specific difference, which the data support well.

The high activity rates at MES may be due in part to the release of labile material (e.g., carbohydrates) during coring and the preparation of incubation vessels (e.g., Howes et al. 1985, *Limnol. Oceanogr.*, 30: 221-227). This phenomenon has been observed when vascular plants are actively remobilizing root/rhizome carbohydrates (e.g., Asaeda et al., 2008, *Hydrobiologia* 607:87–10). It usually occurs in spring when plants are mobilizing stored reserves for early growth, but the current samples were collected in August, so it is unclear if this rapid release of fermentation products might be due to the mobilization of root/rhizome storage carbohydrates. We observed this effect recently (unpublished) in a *Typha* marsh in April in which disturbance resulted in extreme increases in fermentation products in incubation vessels including acetate and a suite of other organic acids, similar to what is reported in the current study. Our interpretation was that newly mobilized organic matter was very labile and rapidly

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stimulated fermentation activity. The resulting stimulation of methanogenesis occurred later leaving a high level of transient intermediates. Again, it would be instructive if the authors included some comments regarding the rates at MES.

The inhibition of methanogenesis by methyl fluoride at the oligotrophic sites (OLI and OMB) was less severe than at MES indicating that methane at the former sites was derived mostly from H<sub>2</sub>/CO<sub>2</sub>. Again, this is similar to what has been reported at some other sites. However, the extent to which H<sub>2</sub>/CO<sub>2</sub> dominates at the OLI and OMB is rather small compared to some other studies, i.e., acetate is still a significant source of methane at the OLI and OMB sites, whereas some other studies reported a nearly complete lack of acetoclastic methanogenesis. The conversion of acetate methane at OLI and OMB is supported by  $\delta^{13}\text{C}$  alpha values of 1.067-1.078, which are high values, but much less than  $\sim 1.09$  that might be expected when all methane is derived from H<sub>2</sub>/CO<sub>2</sub>. However, other studies, have found similar alpha values ( $\sim 1.067$ -1.078) at sites in which very little acetotrophic methanogenesis occurred. This difference may be due in part to the much lower overall rates of activity at these other sites, but more work is needed.

The paper is a well-written and organized account of a study that adds much to a growing field. It has a few typographical errors that can be easily fixed.

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