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Interactive comment on “Free atmospheric CO₂ enrichment did not affect symbiotic N₂-fixation and soil carbon dynamics in a mixed deciduous stand in Wales” by M. R. Hoosbeek et al.

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Reply to referee #1

We would like to thank referee #1 for providing useful comments.

Indeed, next to symbiotic N₂-fixation we should, in a revised version of our manuscript, also discuss heterotrophic N₂-fixation (free-living N₂-fixers). Hofmockel and Schlesinger (2007) hypothesized that heterotrophic N₂-fixation would be enhanced due to increased litter production under elevated CO₂. Increased N availability to plants would, in turn, meet the additional N required to sustain increased NPP under elevated CO₂. They conducted series of experiments in which nitrogenase activity was

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measured in slurries and intact soil cores in response to different levels of substrate, moisture and nutrients. Forest floor and mineral soil samples were obtained from ambient and elevated CO₂ plots at the Duke Forest FACE site. Hofmockel and Schlesinger (2007) did not detect a FACE effect on potential nitrogenase activity and concluded that heterotrophic N₂-fixation was not enhanced in temperate pine forests under elevated CO₂. Based on these results we assume that heterotrophic N₂-fixation at the Bangor-FACE experiment was not affected by elevated CO₂ and did not affect the outcome of our experiment.

The decline of NH₄⁺ under elevated CO₂ was observed in 2008 (Fig 2; Hoosbeek et al., 2010). During this year total soil N did not change as compared to 2007. As mentioned, the decline of NH₄⁺ may have been due to a combination of increased nitrification, microbial activity (N-fixation in SOM) or increased plant uptake under FACE. Indeed, based on the $\delta^{15}\text{N}$ values of *Betula* we may infer that N sources have not changed due to FACE. Moreover, we agree that it would have been interesting to know the $\delta^{15}\text{N}$ values of NH₄⁺ and NO₃⁻ in order to compare these with plant $\delta^{15}\text{N}$ values. Unfortunately, these data are not available.

The classical cascade model in which fresh SOM first enters the 'fast' or 'labile' pool after which it flows into the 'intermediate' pool and eventually end up in the 'slow' or 'stable' pool has been discussed and criticized for a while. The data presented by Lichter et al. (2008) support the conceptual model in which a part of OM entering the soil may be relatively quickly stabilized by, for instance, occlusion in aggregates or adsorption onto mineral surfaces (e.g: Golchin et al., 1994; Jastrow, 1996; Von Lützow et al., 2006; Von Lützow et al., 2008). It would have been interesting to apply this conceptual model to the BangorFACE experiment, but isotopic data of soil fractions are not available.

The Duke Forest is a N-limited system in which adding labile substrate due to FACE will not result in a noticeable priming effect. However, priming may occur in forest FACE experiments established on former agricultural soils. For instance, Hoosbeek

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et al. (2004) observed a priming effect during the second and third year of a FACE experiment with poplar trees established on former agricultural soils in central Italy. This temporal priming effect and the associated loss of older SOM was driven by the experiment, i.e. higher labile C availability due to FACE, and by the change of land use, i.e. going from a high fertility agricultural soil to a forest soil with a declining soil fertility status (Hoosbeek and Scarascia-Mugnozza, 2009). At BangorFACE we also observed that total soil C increased less and total N decreased more under FACE during the second year (Fig 1; Hoosbeek et al., 2010). We infer that soil microbial populations increased under FACE (higher labile C availability) and available N was taken up from this former agricultural soil (lower NH₄⁺ concentration under FACE in 2006). In a next step, or in addition, the extended microbial population decomposed N-rich older SOM making N available to the microbial population and plants. Since this source of N requires more energy, the population will decrease and adjust itself to the new availability of substrate and nutrients. After the increased decomposition of SOM diminished, concentrations of NH₄⁺ and NO₃⁻ will also go down again and adjust to levels that fit the new nutrient status of a young forest soil. This is in line with our observations, i.e. NH₄⁺ decreased after 2007 under FACE, while in the ambient CO₂ plots NH₄⁺ did not decrease yet. While NO₃⁻ decreased both under FACE and ambient CO₂ with lower concentrations under FACE. We postulate that the priming effect and subsequent lowering of soil NH₄⁺ and NO₃⁻ concentrations (not observed yet for NH₄⁺ in the ambient plots) are largely due to the change of land use and that these transitional processes were enhanced under elevated CO₂.

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