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Seasonal effects on nitrate leaching, soil nitrification activity, and seepage-mediated vertical transfer of nitrifiers in a mixed beech forest

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Nitrification in forest soils plays a key role in converting ammonium to nitrate, which can then be leached to deeper soil layers or ultimately to the groundwater. However, seasonal effects on nitrification activity, especially under conditions of summer drought as during the dry summer 2018, are still controversial. In addition, it is virtually unknown if nitrifying microorganisms are also subject to seepage-mediated vertical transfer in these soils. We took advantage of the field infrastructure of the Hainich Critical Zone Exploratory, located in central Germany, providing access to seepage from below the litter layer, 4, 16, and 30 cm soil depth in a mixed beech forest. We hypothesized that (i) soil nitrification activity is highest in summer, resulting in seasonally enhanced nitrate leaching, (ii) increased nitrification activity is linked to increased nitrifier abundances, and (iii) nitrifiers are vertically transferred in these soils.

Samples were obtained in February, May, July, and November 2018, along with sampling of soil from 5 and 10 cm depth. For all four time points, analysis of seepage revealed a general tendency of a decrease of ammonium and an increase of nitrate across the upper 30 cm of the soil. In addition, nitrate fluxes increased by a factor of 10 from February to July with maximum values of 4652 mg m^{-2} over a 14-days sampling intervals while ammonium fluxes decreased by a factor of 3, along with higher soil nitrification activity during the summer with maximum activities of 14,3 nmol g dry $soil^{-1}$ h^{-1} at 5 cm soil depth. Quantitative PCR targeting the *amoA* gene coding for ammonia mono-oxygenase, catalyzing the first step of nitrification, revealed the presence of both ammonia-oxidizing archaea (AOA) and bacteria (AOB), suggesting that both groups could contribute to nitrification activity in these forest soils. Together with an increased soil nitrification activity, AOB abundance showed a 2.5-fold increase in soil from May to July. Both groups of ammonia oxidizers were also found in seepage where they accounted for 0.1 % of the vertically transferred microbiota. Assessment of the total bacterial community in seepage by 16S rRNA gene-targeted amplicon sequencing revealed that *Proteobacteria* and *Acidobacteria* were particularly abundant in the seepage communities. Our results suggest strong seasonal effects on leaching of nitrate and ammonium in a mixed beech forest, which could be linked to seasonally increased nitrification activity but also changes in nitrate sinks. In addition, we demonstrate the seepage-mediated vertical transfer of ammonia oxidizers and other bacterial groups, eventually leading to the export of soil-derived microbiota to deeper soil layers and aquifers.