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Recovery of methane turnover and the associated microbial communities in restored cutover peatlands is strongly linked with increasing *Sphagnum* abundance

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ABSTRACT

Vegetation succession is known to affect carbon-cycling patterns of recovering cutover peatlands, displayed as shifts in emissions of the greenhouse gases CO_2 and CH_4. However, the related plant-microbe interactions are still poorly understood. We aimed to link the recovery of the organisms responsible for CH_4 turnover, the methanogens and the methanotrophs, to the re-vegetation related compositional changes of three functional plant types (*Sphagna*, sedges and shrubs). In peat layers, the *Sphagnum* coverage was the most influential factor for the activity, abundance and community structures of both these microbial groups, demonstrating a succession pattern towards a pristine stage. Analysis of *mcrA* and *pmoA* genes revealed *Methanoregulaceae* and *Methylocystis* as the most dominant methanogens and methanotrophs, respectively. The relatively fast recovery of both CH_4 production and oxidation in the peat layers supports earlier flux based results from these same fen-type peatland sites. In contrast to peat, CH_4 oxidation in living *Sphagnum* mosses appeared to be independent of vegetation succession as CH_4 oxidation potential was similar throughout the succession stages. This indicated that *Sphagnum* may be a valuable CH_4 biofilter especially in the early re-vegetation stages when the oxidation in the peat has not yet recovered. Therefore, we recommend *Sphagnum* transplantation as a tool for climate friendly peatland restoration with faster recovery of the carbon sink function and altered CH_4 emissions.

1. Introduction

Boreal peatlands provide multiple valuable ecosystem services, including climate change mitigation through carbon storage, hydrological buffering and high biodiversity (Chapman et al., 2003; Joosten et al., 2012). Slowly formed peat layers may also be used for energy production and as horticultural growth media. However, utilization of this natural resource leads to the destruction of the functioning ecosystem and formation of large areas of abandoned cutover peatlands. Unlike pristine peatlands, dry, aerobically degrading left over peat together with the lack of photosynthesis usually causes these cutover areas to become a source of carbon to the atmosphere (Nykänen et al., 1996; Waddington et al., 2002; Dixon et al., 2014).

Restoration of cutover peatlands aims to return functions characteristic to pristine ecosystem, such as carbon accumulation as peat and can also be used in reducing the climate warming impact (Wilson et al., 2016). Re-establishment of the original water table and hydrological regime is a key step in creating water-saturated conditions, where decomposition is retarded due to lack of oxygen. With a higher water table (WT) level, typical peatland vegetation returns either spontaneously or through transplantations and leads slowly to the formation of new peat and then again to an even more stable, pristine-state-like hydrology (McCarter and Price, 2015). Pioneering plants provide new carbon substrates and thus enable, after a given lag phase, the recovery of the anaerobic microbial carbon mineralization (Francez et al., 2000; Tuittila et al., 2000a; Andersen et al., 2006). Eventually, the recovery of the climate cooling carbon sink function and the climate warming methane (CH_4) fluxes characteristic to pristine peatlands will follow. A positive carbon balance may often be reached within 3–10 years (since re-wetting) but the site-specific rate and success depends

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on factors such as peatland type, extraction/restoration methods and the climate (Waddington and Day, 2007; Tuittila et al., 1999, 2000a; Soini et al., 2010; Dixon et al., 2014; Strack et al., 2014) and thus e.g. on ombrotrophic bogs, this process may take several decades (Samaritani et al., 2011). In addition, the carbon flux may differ significantly between within-site hydrological microhabitats such as dryer peat and adjacent ditches or ponds (Waddington and Day, 2007; Strack et al., 2014).

Due to its importance for peatland ecosystem recovery, the vegetation is one of the main proxies used in the evaluation of restoration. Without rewetting, dry bare peat would slowly be colonized by pioneer plants, like cotton grass *Eriophorum vaginatum*, haircap moss *Polytrichum strictum* and ericaceous shrubs (Tuittila et al., 2000b; Poulin et al., 2005). Rewetting favors sedges and other fen species that benefit from the high WT (Tuittila et al., 2000b). *Sphagnum* mosses rarely spread spontaneously to the bare peat surface and therefore especially in North America restoration often includes support through *Sphagnum* transplantations (Chimner et al., 2017). However, sites extracted with block-cutting differ from sites extracted with modern milling/vacuum-extraction techniques as the older method leaves the peat layers less compressed and wetter, which favors spontaneous *Sphagnum* recolonization (Lavoie et al., 2003; Yli-Petäys et al., 2007).

The vegetation types differ in their effect on the carbon cycling due to differences in photosynthetic rates, decomposability, rhizodeposition and gas/air conductivity. The fast growing *E. vaginatum* often initiates the carbon accumulation by increasing the CO_2 intake (Tuittila et al., 1999; Marinier et al., 2004). Simultaneously it, like other sedge species such as *Carex* spp, increases CH_4 emissions by providing the CH_4 a direct passage to the atmosphere (Tuittila et al., 2000a; Marinier et al., 2004; Strack et al., 2017) and by excreting substrates for CH_4 production (Ström et al., 2003; Saarnio et al., 2004). As the sedge cover decreases, the carbon sink function may also decrease (Yli-Petäys et al., 2007). *Sphagna* grow slower than the pioneer sedges but are also more recalcitrant to degradation (Clymo and Hayward, 1982), making them the most important plants for the long-term carbon accumulation. Generally, CH_4 emissions are lower from *Sphagnum* dominated sites compared to sites covered with vascular plants (Nykänen et al., 1998). Shrubs have been shown to decrease the decomposition potential on recovering cutover sites (Andersen et al., 2010) but overall, an increase in the shrub cover is believed to have a negative impact on the peatland carbon sink (Bragazza et al., 2015).

The amount of CH_4 emissions is determined by the activity of two types of microorganisms: CH_4 producing archaea (MPA), the methanogens, living mainly below the WT, and CH_4 oxidizing bacteria (MOB), the methanotrophs, functioning in the aerobic surface layers above the WT. Despite the importance of the CH_4 dynamics on the peatland carbon balance, our understanding on the re-vegetation related recovery of these organisms is poor. In addition to direct CH_4 flux measurements, mainly potential activities of MPA and MOB have been analyzed in re-vegetated peatlands (Francez et al., 2000; Glatzel et al., 2004; Basiliko et al., 2007; Strack et al., 2017). Also, *Sphagnum* associated MOB have a major role in peatland CH_4 cycling (Raghoebarsing et al., 2005; Larmola et al., 2010; Putkinen et al., 2014) but the response of *Sphagnum* associated MOB to the restoration succession is unknown.

We characterized the successional change in the CH_4 cycling microbes, MPA and MOB in peat and MOB in the living *Sphagnum* mosses in relation to re-vegetation of rewetted cutover peatlands. For this purpose, we selected three cutover sites representing different stages of re-vegetation from very early plant colonization to an advanced peat accumulating system and one pristine reference site. In addition to the comparison of separate sites, we related the microbial markers to the abundance changes of three main plant functional groups, *Sphagna*, sedges and shrubs. Previous CH_4 emission studies on the same sites showed a rapid response of the CH_4 dynamics to re-vegetation (Tuittila et al., 2000a; Yli-Petäys et al., 2007). Therefore, we hypothesized that the most advanced and the semi-advanced site would harbour MPA and MOB activity and communities similar to the pristine reference site. Due to only partial vegetation cover, a high bulk density, and low CH_4 emissions from similar peat surfaces (Tuittila et al., 2000a; Mahmood and Strack, 2011), the peat layers of the youngest site were hypothesized to have very low microbial activity. As the conditions inside *Sphagnum* mosses are likely to be less affected by the surrounding environment than in peat, the *Sphagnum* associated MOB were hypothesized to experience less changes during re-vegetation than the MOB living in the peat layer.

2. Materials and methods

2.1. Study sites and sampling

We sampled three cutover fens that had been rewetted/restored 2 years, 17 years or 63 years ago and thus represented different stages of vegetation succession. These sites were once bogs but due to the removal of the peat layers, they now received additional water and nutrients from the surrounding area above them, making them ecohydrologically fens. The cutover sites were located on the oldest peat extraction area of Finland, Aitoneva in Kihniö (62°12′N, 23°18′E) and

Table 1
Characteristics of the sampled cutover sites AN2y, AN17y and AN63y and the pristine reference site (PRST).

	AN2y	AN17y	AN63y	PRST
Year of abandonment	2008	1975	1948	–
Year of rewetting/plant introductions	2008/2009	1994	–	–
Method of peat extraction	milling	milling	block-cut	–
Average thickness of the bottom peat left intact after extraction	25 cm	1 m	na	85 cm (total)
Water table level during sampling (cm relative to the peat surface, mean ± s.e.)	−12 ± 0	−4 ± 0	−16 ± 1.2	−11 ± 0.7
pH[a] (mean ± s.e.)	−15 cm: 5.27 ± 0.21 −25 cm: 5.48 ± 0.06	−15 cm: 4.46 ± 0.08 −25 cm: 4.29 ± 0.05	−15 cm: 4.01 ± 0.02 −25 cm: 4.19 ± 0.02	−15 cm: 4.42 ± 0.01 −25 cm: 4.42 ± 0.03 bottom: 4.92 ± 0.17
Peat bulk density in the -25 cm layer (g $dm^{3\ -1}$, mean ± s.e.)	130 ± 4.5	88 ± 4.3	42 ± 8.2	33 ± 2.1 (110 ± 26 for bottom)
Vegetation (%, mean ± s.e.)	*Sphagnum* 36 ± 19 Sedges 9.7 ± 5.4 Shrubs 21 ± 5.2 other: *Polytrichum* sp. 8.3 ± 5.5 *Marchantiophyta* 4.0 ± 1.7	*Sphagnum* 90 ± 1.7 Sedges 19 ± 6.7 Shrubs 0	*Sphagnum* 98 ± 1.5 Sedges 3.7 ± 2.3 Shrubs 7.0 ± 4.4	*Sphagnum* 100 Sedges 10 ± 2.7 Shrubs 6.3 ± 3.5

[a] Measured from the flasks used in the CH_4 production potential analysis (after the analysis).