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Running title: CH_4 production and oxidation processes

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Abstract

Mires, especially sedge dominated fens, are sources of the greenhouse gas CH_4. Climate change scenarios predict a lowering water table (WT) in mires. To study the effect of WT drawdown on CH_4 dynamics in a fen ecosystem, we took advantage of a WT drawdown gradient near a ground water extraction plant. Methane fluxes, CH_4 production and oxidation potentials, were related to microbial communities responsible for the processes in four mire locations (wet, semi-wet, semi-dry and dry). Principal component analyses (PCA) performed on the vegetation, pH, CH_4 and WT results clearly separated the four sampling locations in the gradient. Long-term lowering of WT was associated with decreased coverage of *Sphagnum* and aerenchymatic plants, decreased CH_4 field emissions and CH_4 production potential. Based on *mcrA* T-RF the methanogen community structure correlated best with the methane production and coverage of aerenchymatic plants along the gradient. Methanosarcinaceae and Methanocellales were found at the pristine wet end of the gradient, whereas the Fen cluster characterized the dry end. The methane-oxidizing bacterial (MOB) community consisted exclusively of *Methylocystis* bacteria, but interestingly of five different alleles (T, S, R, M, and O) of the particulate methane monooxygenase marker gene *pmoA*. The M allele was dominant in the wet locations, and the occurrence of alleles O, S and T increased with drainage. The R allele that characterized the upper peat layer correlated with CH_4 oxidation potential. These results advance our understanding of mire dynamics after long-term WT drawdown and of the microbiological bases of methane emissions from mires.

Introduction

In the Northern hemisphere mires extend over vast areas that typically are water-saturated ecosystems where a part of the plant litter production avoids aerobic decomposition and accumulates as peat. The high water table (WT) creates anaerobic microbial habitats where methanogenesis occurs (Rydin *et al.* 2006). Hence boreal mires act as methane (CH_4) sources into the atmosphere. Due to climate change, a growing attention has been devoted to these areas, where over one-third of the global terrestrial carbon is stored (Gorham, 1991). The rise of earth temperatures may have profound effects on the microbial life of these ecosystems and thus on microbially-mediated gas emissions (IPCC 2007).

Methane, which is a 25 times more powerful greenhouse gas than carbon dioxide, is produced microbiologically in the final stage of anaerobic degradation of organic matter (Whalen, 2005). Methane is solely produced by methanogens that are classified into Euryarchaeota. Methanogens are highly diverse constituting of four classes and six orders of archaea. Methane-producing archaeal communities have been described from various mire ecosystems (Edwards *et al.* 1998; Basiliko *et al.* 2003; Galand *et al.* 2003; Galand *et al.* 2005; Galand *et al.* 2005; Juottonen *et al.* 2008; Putkinen *et al.* 2009) including a range of nutrient conditions (Juottonen *et al.* 2005) and mire successional stages (Merilä *et al.* 2006). Methanogens of Methanosarcinales, Methanomicrobiales, Methanobacteriales and Methanocellales (Rice cluster I) have frequently been detected.

The CH_4 emissions are, however, dependent on the activity and abundance of methane-oxidizing bacteria (MOB) that are able to oxidize CH_4 to CO_2 in aerobic conditions (Hanson and Hanson 1996). The aerobic layer of peat is roughly defined by the WT position (Bubier & Moore, 1993). Low emissions in mires can occur when the CH_4 never reaches the atmosphere because it is consumed by MOB in the uppermost aerobic layer (Whalen, 2005). In this way the CH_4 flux to

the atmosphere is the sum of the functionally opposite actions of archaea and bacteria involved in the CH_4 cycle. The highest MOB activity in mires is observed just above the WT, where CH_4 and oxygen levels are adequate for CH_4 oxidation (Sundh *et al.* 1994). While estimates of the oxidation efficiency of the produced CH_4 in different mires vary considerably, estimates of between 20% in *Carex* dominated fens (Popp *et al.* 2000) and 78% in *Sphagnum* dominated bogs (Yavitt *et al.* 1988) have been given. The latter phenomena is explained by the fact that MOB inhabit *Sphagnum* species providing the plant CO_2 through CH_4 oxidation under wet conditions (Raghoebarsing *et al.* 2005; Larmola *et al.* 2010).

MOB are traditionally divided into two taxonomic groups, type I and II, within the Proteobacteria. Type I MOB includes genera like *Methylobacter* and *Methylomicrobium*, which belong to the Gammaproteobacteria. The type II MOB, *Methylocystis*, *Methylosinus*, *Methylocella* and *Methylocapsa* belong to the Alphaproteobacteria (Hanson & Hanson 1996; Dedysh, 2009). These types differ in their carbon assimilation pathway, phylogenetic affiliation, and intracellular membrane arrangement.

The methanogenic and methanotrophic communities in Finnish mires have been studied by molecular genetic methods. Methanogens were analyzed by using both functional, *mcrA* (Galand *et al.* 2002a), and phylogenetic 16S rRNA marker genes (Galand *et al.* 2003). MOB have been studied by functional (*pmoA*) marker gene analysis in a pristine and drained fen and bog within a mire complex (Jaatinen *et al.* 2005). The fingerprinting of Finnish fen MOB was improved by modifications of the *pmoA* reverse primer for DGGE analysis protocols (Tuomivirta *et al.* 2009). Methanogens and methanotrophs in mires have, however, rarely been analyzed within the same study (McDonald *et al.* 1999).

The CH_4 turnover in mires is highly dependent on the ecohydrological conditions. In climate change scenarios where the raise of temperature is 3 $^{\circ}$C, the summertime WT in northern

mires is expected to drop 10-20 cm (Gorham 1991; Roulet *et al.* 1992; Gitay *et al.* 2001). A WT drawdown of that magnitude (15 cm) has occurred in the northern Suonukkasuo fen where a groundwater extraction plant has generated a WT gradient that has gradually changed part of the wet fen into a forested peatland (Jaatinen *et al.* 2008). Such a persistent change in the WT influence the plant community structure (Weltzin *et al.* 2000; Weltzin *et al.* 2003) that may lead to complete replacement with species better adapted to the new conditions (Laine *et al.* 1995; Strack *et al.* 2006).

The WT gradient was studied combining methane gas flux measurements, environmental data and measurements of microbial communities responsible for CH_4 turnover. This gradient study prompted the formulation of hypotheses concerning CH_4 turnover in relation to detected microbial communities, namely: (1) as the WT drawdown affects the plant cover and stimulate tree growth, the CH_4 emissions are reduced in the resulting dryer conditions; (2) the emissions in WT drawdown conditions decrease primarily as a consequence of decrease in potential CH_4 production and simultaneous decrease in diversity of methanogens; (3) the potential CH_4 oxidation decreases when there is less CH_4 available; (4) the depth is an important factor determining microbial community structure, both that of methanogens and of methanotrophs; (5) there are specific types of methane-producing archaea and MOB that are favored by the WT drawdown.

Materials and methods

Experimental site and sampling

The study site, Suonukkasuo, is a mesotrophic pine fen located in Rovaniemi, northern Finland (66°28′N, 25°51′E) within the aapa mire zone. Mesotrophic pine fens (RhSR in the Finnish mire

site-type nomenclature of Laine and Vasander (2005)) are typically sites where wet lawns and drier hummocks form a mosaic-like vegetation pattern. A groundwater extraction plant on an esker bordering the mire downstream has affected the WT at the study site since 1959, resulting in a clear hydrological gradient where the pristine wet fen (location S4) becomes semi-wet (location S3), semi-dry (location S2), and finally a dry pine dominated mesotrophic peatland forest (MtkgII, location S1).

The average long term water-table (WT) values for the ground-frost free periods of the years 2001–2003 and 2005 expressed as distances from soil surface were 26, 21, 15 and 9 cm for the locations S1 to S4, respectively. The differences in WT between locations were statistically significant, except between locations S1 and S2 ($p=0.066$). The first- and third-quartile ranges of the WTs were 21–30, 13–30, 10–21 and 5–13 cm for the locations S1 to S4, respectively. The momentary WT at the time of sampling in 2006 was 32, 50, 47 and 23 cm, respectively. The hydrological gradient is reflected in vegetation composition as reported in Jaatinen *et al*. (2008).

Sampling was conducted in September 2006. Intact triplicate peat cores were taken from the four locations using a corer (size 4 x 6 cm) to a depth of 70 cm. Depth samples were prepared by dividing peat cores at 10 cm intervals. The samples between 0 - 50 cm depth were used for estimation of the methanotroph activity and community analyses and the samples between depths 20 - 70 cm for the respective analyses for methanogens. For convenience we call the samples between 0 to 10 cm as 0 cm, between 10 to 20 cm as 10 cm and so on. To measure the peat dry weight subsamples were kept at 105 $^{\circ}$C for over 12 h and subsequently weighted.

Field CH_4 measurements

Gas efflux measurement plots (n = 3) were installed at each location in June 2001 (Dahlin *et al*., 2003). Collars made of metal tubes (diameter 31.5 cm) were inserted 15-30 cm deep in peat. An

aluminum groove was attached on the top of each collar. A closed chamber (height 30 cm) equipped with a fan was placed on the groove filled with water before sampling to seal the chamber. At each sampling occasion, four successive samples of 30 ml were taken from the chamber with plastic syringes during a 35-minute measurement period at 10 minute intervals (5, 15, 25, 35 minutes). The syringes were then taken to laboratory and analyzed for CH_4 concentrations within 24 hours from sampling by a gas chromatograph equipped with a FI detector. CH_4 fluxes were calculated from a linear change of CH_4 concentrations during the sampling period of 35 minutes. Obvious ebullition events were deleted from the data.

Measurements of potential CH_4 production and oxidation

Measurements of potential CH_4 production and oxidation (4.13 μmol in 125 ml infusion bottle) with incubation at 15 ^{o}C were carried out as described in Juottonen *et al.* (2008) and Jaatinen *et al.* (2005), respectively.

Analysis of methanogen communities by *mcrA*-TRFLP

DNA for methanogen community analysis was extracted from 0.25 g of wet mass peat with PowerSoil DNA Isolation Kit (MoBio Laboratories). Methyl coenzyme M reductase (*mcrA*) gene fragments were amplified with the primers of Luton *et al*. (2002). The reactions (50 μl) contained 1 × PCR buffer with 2 mM $MgCl_2$ (Biotools), 200 μM dNTPs, 0.4 μM of both primers, 1U of DNA polymerase (Biotools), and 1-2 μl of undiluted DNA extract as template. The reaction conditions were initial denaturation (94 °C, 3 min) followed by 31 cycles of 94 °C 45 s, 52 °C 1 min, 72 °C 1 min 30 s, and a final elongation (72 °C, 7 min). In PCR for T-RFLP, the forward primer was 5'-labelled with 6-carboxyfluorescein (FAM).

For T-RFLP, approximately 25-50 ng of PCR products was digested with 3 U of *Msp*I (Fermentas) at 37 °C overnight. The T-RFLP analysis was carried out as described in Juottonen *et al.* (2008). The range of fragment lengths included in the analysis was 75 to 500 bp. Minimum peak height threshold was 100 fluorescence units. Results are presented based on relative peak area. For identification of T-RFs, three clone libraries (S4 20 cm; S2 30 cm; S1 50cm) were constructed as in Juottonen *et al.* (2008). Inserts from clone colonies were re-amplified with *mcrA* primers (33-54 clone per library) and screened by RFLP with *Msp*I. From RFLP groups with > 5 clones, 2-5 clones from each group were sequenced, and from groups with < 5 clones, one member was sequenced as in Juottonen *et al.* (2008). Terminal restriction fragments were identified based on *in silico* digestion of the sequences and T-RFLP analysis of clones.

Investigation of MOB by DGGE analysis of the *pmoA* marker gene

DNA for methane-oxidizing bacteria (MOB) community analysis was extracted from ca. 0.5 g wet mass peat using the FastDNA kit for soil (MP Biomedicals, Ohio, USA) according to Yeates & Gillings (1998) modified as in Tuomivirta *et al.* (2009). A region of subunit α of particulate methane monooxygenase (*pmoA*) was PCR targeted with the A189f/A621r with a GC-clamp attached to the reverse primer (see Tuomivirta *et al.* 2009 for details). Also broad specificity A189f/A682r (Holmes *et al.* 1995) primers were applied for reference (Tuomivirta *et al.* 2009).

Fingerprinting of the MOB diversity was performed in a denaturing gradient gel electrophoresis (DGGE) described in Jaatinen *et al.* (2005). The DGGE gel photographs were screened for the presence (1) or absence (0) of *pmoA* bands using the AlphaImager 2.1 program of the AlphaDigiDoc gel documentation system (Alpha Innotech Corp., CA. US). A binary matrix was generated with this data using only the positions of successfully sequenced bands.

Single DGGE bands of interest were excised from the gel, purified and sequenced as described in Tuomivirta *et al.* (2009) and Larmola *et al.* (2010).

Sequence analysis and phylogeny

mcrA and *pmoA* sequences were compared to database sequences with BLAST (Altschul *et al.* 1997) analysis of the National Center for Biotechnology Information (NCBI). Deduced amino acid sequences of *mcrA* together with selected reference sequences were aligned with ClustalW (Larkin *et al.* 2007). Suitable evolutionary model was selected with ProtTest (Abascal *et al.* 2005). Maximum likelihood trees were constructed with PhyML (Guindon & Gascuel 2003) with model LG+G+F. Bootstrap values were generated from 100 replicates in PhyML. Pairwise distance calculations of nucleotide *pmoA* sequences were analyzed with MEGA 4.0 (Tamura *et al.* 2007). The *mcrA* and *pmoA* sequences have been submitted to the EMBL database under accession nos. FN564006-FN564029 (*mcrA*) and GQ279342-GQ279346 (*pmoA*).

Statistical evaluation

Methane fluxes between locations were compared by one-way analysis of variance (ANOVA). Significance of pairwise differences was assessed by Tukey test. The effect of categorical variables depth, location and their interaction on potential CH_4 production and oxidation were compared with generalized linear models (GLM). Tukey post hoc test was applied to determine which pairs of means differ significantly. Because location and depth had significant interactions, ANOVA followed by Tukey test was performed separately for each depth. Level of significance in all statistical analyses was P=0.05.

We applied principal component analysis (PCA) to compare the four locations based on their environmental variables, i.e., long-term and momentary WT, CH_4 field emission and

oxidation and production potential, coverage of *Sphagnum* and aerenchymatic plants, and pH. To examine variation in methanogen and MOB communities, we first applied detrended correspondence analysis (DCA). Based on the DCA showing rather small compositional variation, (ter Braak & Prentice 1988) we applied redundancy analysis (RDA) to test which environmental variables best explained variation in methanogen and MOB communities. The significance of the above listed explanatory factors was assessed using Monte Carlo permutation test. The multivariate analyses were carried out with Canoco for Windows (ter Braak & Smilauer 2002).

Results

Methane emissions

In the years (2001—2004) preceding the sampling for microbial community analysis, the CH_4 emissions from location S4, were substantial, averaging 73 mg m^{-2} day^{-1} (Fig. 1). As a result of WT drawdown, the emissions were much smaller in the locations S3 (22 mg m^{-2} day^{-1}), S2 (0.9 mg m^{-2} day^{-1}) and S1 (1.9 mg m^{-2} day^{-1}; Fig. 1). Similarly to long term WT the emissions differed significantly between all pairs of locations (P=0.001—0.004) except for locations S2 and S1.

Methane production and oxidation potential

The wettest location S4 had the highest CH_4 production potential at depths from 20 to 50 cm (P=0.001—0.036, Fig. 2) in correspondence with the flux rates recorded in earlier years. The S4 production was 43.2 nmol g dry $weight^{-1}$ day^{-1} compared to 0.4 (S3, p=0.005), 4.0 (S2, p=0.010) and 0.4 (S1, p=0.005) at the depth of 20 cm. The production potential of the three other dryer

locations was much lower and did not differ between the locations at any depth. The laboratory measurements showed that location and depth significantly affected the CH_4 production potential through the WT gradient ($p<0.001$).

The highest methane oxidation potential was observed in location S3, 561.2 nmol g dry weight^{-1} day^{-1}, but the sampling depth influenced the oxidation potential ($p=0.004$) in addition to location ($p=0.033$) (Fig. 2). In the top layer the potential was significantly higher in S3 than in S2, 62.2 nmol g dry weight^{-1} day^{-1} ($p=0.042$) and S1, 28.9 nmol g dry weight^{-1} day^{-1} ($p=0.031$) and second highest in S4, 177,7 nmol g dry weight^{-1} day^{-1}. The dry S1 location had the lowest potential oxidation rate. The oxidation potential did not differ between locations in deeper peat layers.

Environmental factors characterizing the WT gradient

Principal component analyses (PCA) performed on the vegetation, potential CH_4 production and oxidation, CH_4 field emissions, pH, and long term and momentary (date of sampling) water-table depth clearly separated the four sampling locations along the first principal axis (PC1 in Fig. 3a). With the provided measurements the second principal axis (PC2) separated all samples according to depth. Increase in long term WT, coverage of *Sphagnum* and aerenchymatic plants, high CH_4 field emissions together with CH_4 production potential characterized the pristine end of the PC1 (Fig. 3b). Decreasing momentary WT and CH_4 oxidation potential together with increasing pH characterized the peat layers with increasing depth along PC2.

Microbial communities involved in the CH_4 cycle along the gradient

T-RFLP fingerprinting of *mcrA* representing methanogens resulted in twelve T-RFs (Table 1). In the RDA T-RFs 468 and 471 were combined since the large size of T-RFs prohibited reliable

distinction between them. According to RDA the methanogen community structure showed highest correlation with methane production and coverage of aerenchymatic plants along the gradient (RD1 in Fig. 4a-c). The second RDA axis showed that the pH influenced the community structure according to depth. The first RDA axis separated the four locations when the methanogen communities at different depths were analyzed together with environmental factors (Fig. 4b). The top layers were most variable through the gradient, but deeper down they became more alike.

In the wet end of the gradient with higher CH_4 production potential the methanogen diversity was highest with 8 to 10 T-RFs. The locations S2 and S1 sampled from three depths showed lower diversity with 6 T-RFs.

The occurrence of the T-RFs 115 bp (Methanocellales), 272 bp (putatively Methanosarcinaceae) and 298 bp (Methanosarcinaceae) correlated most strongly with the wet location S4 (Fig. 4c, Fig. 5). In addition to identified methanogens, T-RF 193 bp affiliated with a cluster with no cultured representatives in the phylogenetic tree (Fig. 5). The T-RF 278 bp, representing Methanomicrobiales-associated Fen cluster, was one of two T-RFs prone to the dry end of the gradient despite of overall decrease in methanogen groups along the gradient.

In analysis of MOB communities, the broad specificity *pmoA* primer set A189f/A682r did not produce correct amplicons (Tuomivirta *et al.* 2009). Five different *pmoA* alleles (T, S, R, M, and O), all *Methylocystis* spp according to sequence comparisons, could, however, be isolated from the gradient by PCR-DGGE and sequencing with the A189f/A621r primer set (Table 2). RDA (Fig. 6a) of the *pmoA* alleles showed that their occurrence was best correlated with CH_4 oxidation potential, pH and vegetation. Similarly to methanogen communities (Fig. 4a) the locations from wet to dry were separated along the first RDA axis, and the second RDA axis characterized the depth of the peat (Fig. 6b). The two wettest locations had similar MOB

communities in their whole profile (Fig. 6b). The deep layers (30 - 40 cm) of the locations S4, S3 and S2 were also characterized by a similar MOB. The MOB community of the dry location S1 differed from the other sites. The M allele, dominating the wet end of the gradient (Fig. 6a) was identical to a previously detected Finnish fen sequence FJ930087 (Tuomivirta *et al.* 2009) and was most different from the other alleles with 11 to 12 point mutations. The R allele inhabiting the wetter part of the gradient was identical to sequences FJ930090 and FJ930091 of a Finnish fen (Tuomivirta *et al.* 2009), AY309209, a North-American fen (Nold, personal communication), and the sequence GQ121280 (Larmola *et al*. 2010) occurring in methane oxidizing community within *Sphagnum* mosses. Drying correlated with increasing abundances of alleles O, S and T. The R allele dominated the pristine location and the upper layer of the peat (Fig. 6a).

Discussion

Generally the study supports the five hypotheses concerning CH_4 turnover in relation to detected microbial communities along WT drawdown gradient. The study of greenhouse gas turnover along a peatland WT gradient showed that drying appeared to cause a dramatic reduction in CH_4 emissions and in potential CH_4 production in agreement with our hypotheses 1 and 2. Despite more oxic conditions the methane oxidation also decreased substantially along the gradient. The phenomena supported our third hypothesis on the superior role of the availability of CH_4 for the potential CH_4 oxidation over the direct WT control. In agreement with the fifth hypothesis drying decreased the diversity of the methanogen communities and brought forth an adapted group of archaea, the Fen cluster methanogens, which was characteristic of dryer conditions. As hypothesized (hypothesis 4) the depth appeared to be an important factor for the community structure of methanogens and of methanotrophs. The depth effect in WT drawdown brought

about a peculiar shift in methanotroph populations showing specific *pmoA* allele types of *Methylocystis* bacteria distributed to specific locations in the gradient. Previously, at the same site, the CO_2 flux increased from location S4 to S1 and fungi and bacteria benefited from the WT drawdown whereas actinobacteria did not (Jaatinen *et al*. 2008).

Separation of the four locations along the WT gradient and the sampling depths (peat layers) was clear and could statistically be explained by eight variables. The WT gradient was best described in PCA by coverage of aerenchymatic plants, high CH_4 emissions at the wet end, coverage of *Sphagnum* moss and CH_4 production potential in this order. The CH_4 oxidation was reduced as a consequence of WT drawdown but not as clearly as the production. This may well be indicative of the difference in the two processes of production (anaerobic) and the following consumption (aerobic) of CH_4. The CH_4 oxidation potential indeed correlated positively with the WT of the time of sampling and the coverage of *Sphagnum*, whereas production correlated with other variables like pH and emissions (Fig. 3b). The high oxidation in wet locations seemed to be coupled with high CH_4 production potential. When more CH_4 is potentially produced by methanogenic archaea, more is simultaneously oxidized by the methanotrophic bacteria.

Very recently methane oxidation was reported from 23 different *Sphagnum* species originating from a Finnish boreal mire site and the activity was related to a *Methylocystis* signature similar to the R allele of this study (Larmola *et al*. 2010). The correlation of the oxidation potential with *Sphagnum* coverage might be a direct regulation instead of a covariation with more pristine conditions.

The methanogen community structure according to T-RFLP analysis changed with decrease in diversity along the gradient. The maximum CH_4 production potential for each vertical peat core correlated with the diversity of methanogens so that decreasing production was reflected in decreasing diversity. WT gradients have mainly been studied in meso- and

microcosms (see, however, Talbot *et al*. 2010), and as far as we know reports from natural gradients combined with microbial community analysis of the CH_4 are lacking. In agreement with our results from the gradient, experimental field studies in Canada (Strack *et al*. 2004) and in Ireland (Laine *et al.* 2009) have shown decrease in CH_4 emission as a result of lowered WT. Short term effects of WT on carbon balance in pore water from acidic, oligotrophic mire have been reported from a mesocosm study (Blodau *et al.* 2004) where decreased CH_4 production and emission was reported as a result of lower WT. Short term effect of drought (4 week) on the methanogen community structure in a bog and a fen mesocosm could not be observed (Kim *et al.* 2008), but a significant decrease in the *mcrA* abundance was detected in the fen mesocosm.

Sampling depth has been shown to affect the methanogen community structure in mires (Galand *et al.* 2002b) where the changing conditions with depth (oxygen and quality of growth substrates for the microbes active in methanogenesis) will select for methanogen groups. The RDA analysis of the methanogen communities together with environmental factors showed nicely how the locations differ at the surface, but become much alike in the deepest sampling layer where the conditions in the gradient apparently are more similar (Fig. 4b). The discrepancy of location S2 to the general trend may well be explained by larger pH variation at this location (results not shown) and lowest emissions telling that this location is the most disturbed one in the gradient.

The methanogen group most strongly associated with the pristine end of the gradient was Methanosarcinaceae. In the drier locations, Methanosarcinaceae T-RFs were less abundant or absent. This finding was surprising considering that many members Methanosarcinaceae are versatile methanogens that are able to use several different substrates for growth (Garcia *et al.* 2000). They also possess enzymes for detoxification of oxygen, which could favor their occurrence in the dry end of the gradient, which is the opposite of what was observed. Additional

data, such as availability of methanogenic substrates along the gradient, would be required to better explain the disappearance of Methanosarcinaceae at the dry end. A minor group also found primarily at the wet end was an uncultured cluster (Mx cluster in Fig. 5) which has been detected in other wetland ecosystems (Juottonen *et al.* 2005; Castro *et al.* 2004; Smith *et al.* 2007) and in animal feces (Ufnar *et al.* 2007), and it has been proposed to form an entirely new order of methanogens (Mihajlovski *et al.* 2008). Only two methanogenic groups were typical to the dry end of the gradient, and they were affiliated with the Fen cluster which has commonly been found in Finnish mires (Juottonen *et al*. 2008). One member of this cluster, *Methanoregula boonei*, was isolated from an acidic bog and has the lowest pH optimum known for a methanogen (Bräuer *et al.* 2006). It is tempting to say that the Fen cluster/Methanoregula-type hydrogenotrophic methanogens are well adapted to the conditions of boreal fens characterized by changing WT and low pH.

All detected MOBs were by *pmoA* sequence analysis found to be of the *Methylocystis* genus, but differed by minute changes, point mutations, in the gene (Table 2). This suggests a phylogenetically very narrow MOB population of this fen. RDA showed that the occurrence of the five alleles was clearly dependent on location and peat layer in the gradient. It can be assumed that before WT drawdown, when the whole site was botanically a homogenous fen, all the locations had a similar MOB population with dominating alleles R and T in surface peat and then changing to T and M alleles in the deeper peat. Continual drainage of the fen has a selective pressure on MOB. The R allele was lost upon drying since methanotrophy in *Sphagnum* mosses is only related to high WT (Larmola *et al*. 2010). For reasons we do not know the most different M allele, in respect to other ones, disappears in WT drawdown and is replaced by the O and S alleles through out the peat layer. The S allele could originate from the generalist T through a

single silent point mutation in *pmoA*. The O has probably been introduced to the location as it contains four silent point mutations in respect with S.

Methylocystis was a dominating genus also in Russian and German bogs, which showed that 60-95% of MOB belonged to type II with *Methylocystis* being the dominant genus (Dedysh *et al.* 2003). A study, in which stable isotope probing (SIP) with PLFA, mRNA and microarray methods were used, revealed the dominance of type II genera *Methylocystis* and *Methylosinus* in blanket peat samples from England (Chen *et al.* 2008). Bearing in mind that also *Sphagnum* species possess *Methylocystis* (Larmola *et al*. 2010), one can say that it most likely dominates in northern peatlands.

With regard to climate change effect on boreal fens, the average drop in WT between location S4 (wet fen) and location S3 (semi-wet) was ca. 6 cm. This was accompanied by a statistically significant drop in potential CH_4 production together with a reduction in emissions from previous years. The potential oxidation changed in the other direction with increasing amounts of CH_4 oxidized at location S3 as compared to location S4. This would indicate that already a small, 6 cm, WT drawdown will change the CH_4 turnover. The methanogen community structure most clearly shifted between locations S3 and S2 and the distribution of the methanotrophic allele types is also changing most clearly in this part of the gradient. It can, however, be stated that as the underlaying microbial communities are changing with a 12 cm drop in WT their activity is already altered after the 6-cm initial drop in WT.

Comparison of behavior of methanogens and methanotrophs in the WT gradient using CH_4 production and oxidation measurements showed a difference in sensitivity to changing conditions. Much of the methanogenic activity is evidently lost already in the semi-wet location S3 and after that decreases drastically, whereas the methanotroph potential activity is high in the wet locations and decreases much less in the dryer locations. This can be explained by more oxic

conditions that should favor these aerobic bacteria. The drop is best explained by the lack of substrate; CH_4.

As a general conclusion, we have been able to show that even a minor WT drawdown, mimicking the climate change impact affects the dynamics of CH_4 production and oxidation in a mire. This study thus shows how ecohydrology shapes the dynamics of mires changing the plant community structures, and has a drastic effect on the microbial communities that are the basis for the environmentally important function of greenhouse gas turnover.

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Table 1 Identification of T-RFs detected in analysis methanogens in the mires forming a water table (WT) gradient. The occurrence of PCR-T-RFLP detected methanogens in the WT gradient formed by the four locations S4-S1 and the relative abundance of T-RFs in each location.

T-RF (bp)	Methanogen group	Occurrence of T-RF (%)			
		S4	S3	S2	S1
105	no sequences	3	2	-	-
115	Methanocellales	6	4	-	-
193	Mx cluster	5	1	2	-
243	Methanosarcinaceae	-	-	-	1
251	Methanocellales	13	18	7	6
272	Methanosarcinaceae[a]	14	6	5	1
278	Fen cluster (Methanomicrobiales)	31	41	70	69
293	Fen cluster (Methanomicrobiales)	16	15	3	14
298	Methanosarcinaceae	2	-	-	-
468	Methanobacteriaceae	9[b]	13[b]	13[b]	8[b]
471	Fen cluster (Methanomicrobiales)				
489	Methanosarcinaceae	1	-	-	-
		wet	semi wet	semi dry	dry

[a] No sequences were detected in this study but identification is based on sequences from other mire sites (Juottonen *et al*., unpublished data).

[b] Includes T-RFs 468 and 471 bp.

Table 2 Number of point mutations (integers) between different *pmoA* allele sequences and standard errors (in parenthesis) of pairwise distance calculations. Accession numbers in Genbank are GQ279342 (T), GQ279343 (S), GQ279344 (R), GQ279345 (M) and GQ279346 (O), respectively.

Sequence of *pmoA*					
O	5	4	7	11	
M	12	11	12		
R	2	3			
S	1				
T					
	T	S	R	M	O
T		[0.00198]	[0.00286]	[0.00977]	[0.00513]
S	0.00122		[0.00376]	[0.00918]	[0.00455]
R	0.00244	0.00367		[0.01011]	[0.00648]
M	0.01518	0.0139	0.01522		[0.00944]
O	0.00616	0.00492	0.00867	0.01382	

Fig. 1 Average methane emission 2001-2004 from the four locations forming a water table (WT) gradient, from wet fen location S4 to dry forested location S1.

Fig. 2 (a) The potential methane production, (b) the potential methane oxidation in the four locations S4 - S1. The depths are given as distance from the surface of the mires.

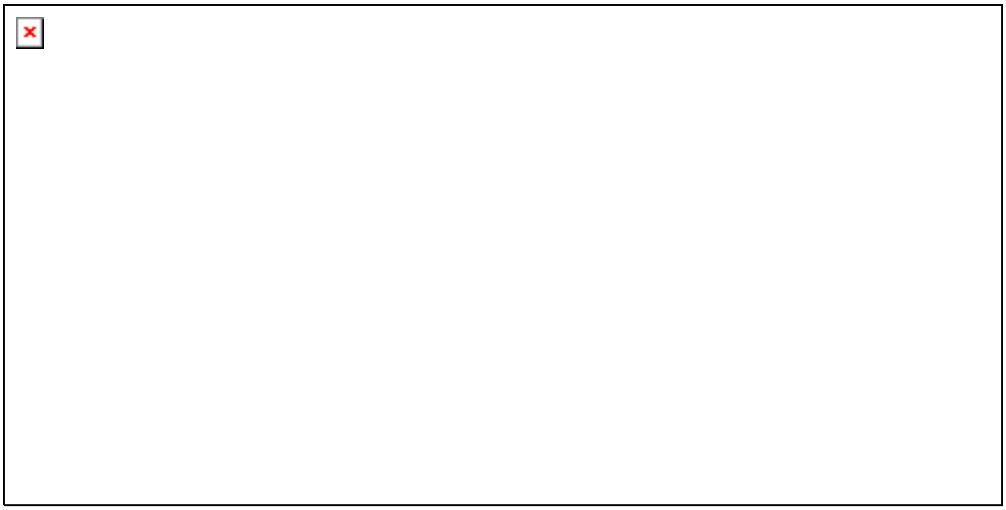
Fig. 3 (a) Principal component analysis (PCA) of environmental factors, emissions, vegetation, water table (WT) for locations S4 to S1 coupled with CH_4 production and oxidation. The direction of the arrow describes the correlation between measured factors. The length of the arrow gives the degree of explanation of each variable in the ordination. The two first principal components together explained 55.3% of the variation. Eigenvalues of the first and second axis were 0.366 and 0.186, respectively. (b) Score plot of the PCA.

Fig. 4 (a) Redundancy analysis (RDA) of statistically significant environmental factors and methanogen communities. The length of an arrow describes the degree of explanation of environmental factor. The first two axes explained 28.0% of the compositional variation. (b) RDA of methanogen phylotypes (T-RFs) at different depths and environmental factors. (c) RDA of T-RFs detected in the water table (WT) gradient. The length of arrow describes how well the phylotype correlates with locations in the gradient.

Fig. 5 Maximum likelihood tree of partial *mcrA* sequences (128 aa) from Suonukkasuo fen clones (in bold) and reference methanogen sequences. Size of terminal restriction fragment is indicated for each group. Scale indicates 10% sequence divergence. Nodes with bootstrap values >95% are marked with filled circles and >75% with open circles. The tree was rooted with *Methanopyrus*

kandleri. In bold sequence names Suon1, Suon2 and Suon4 refer to locations S1, S2 and S4 of the water table gradient.

Fig. 6 (a) RDA of methanotoph *pmoA* alleles showing correlation strength (length of arrow) with statistically significant environmental factors. The first two axes explained 47.1% of the compositional variation. (b) The change in methanotroph communities according to depth and environmental factors picturing the WT gradient.



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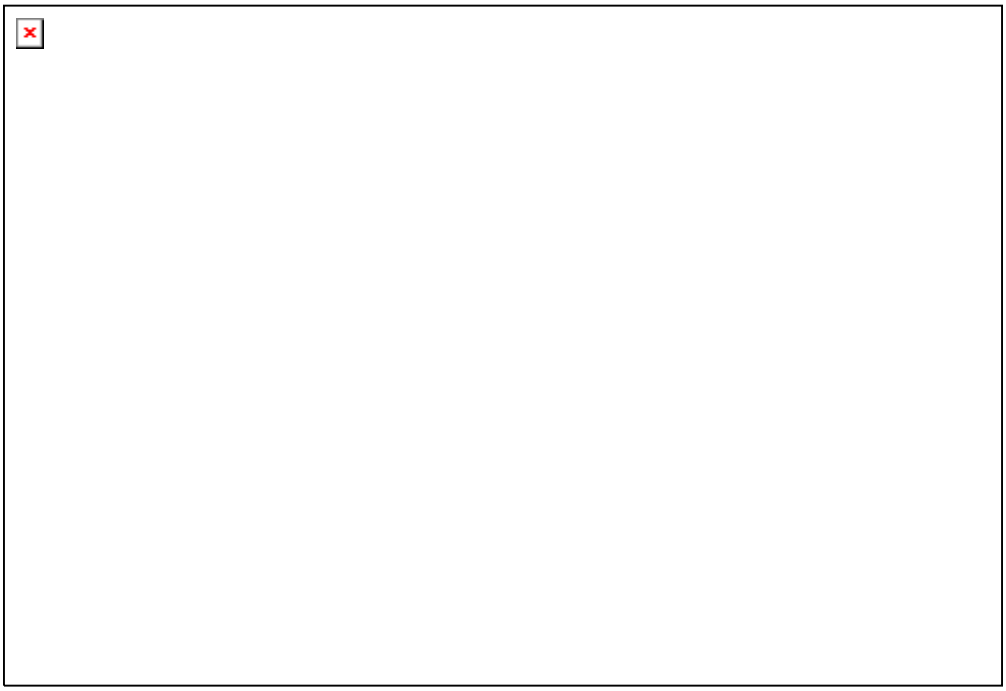

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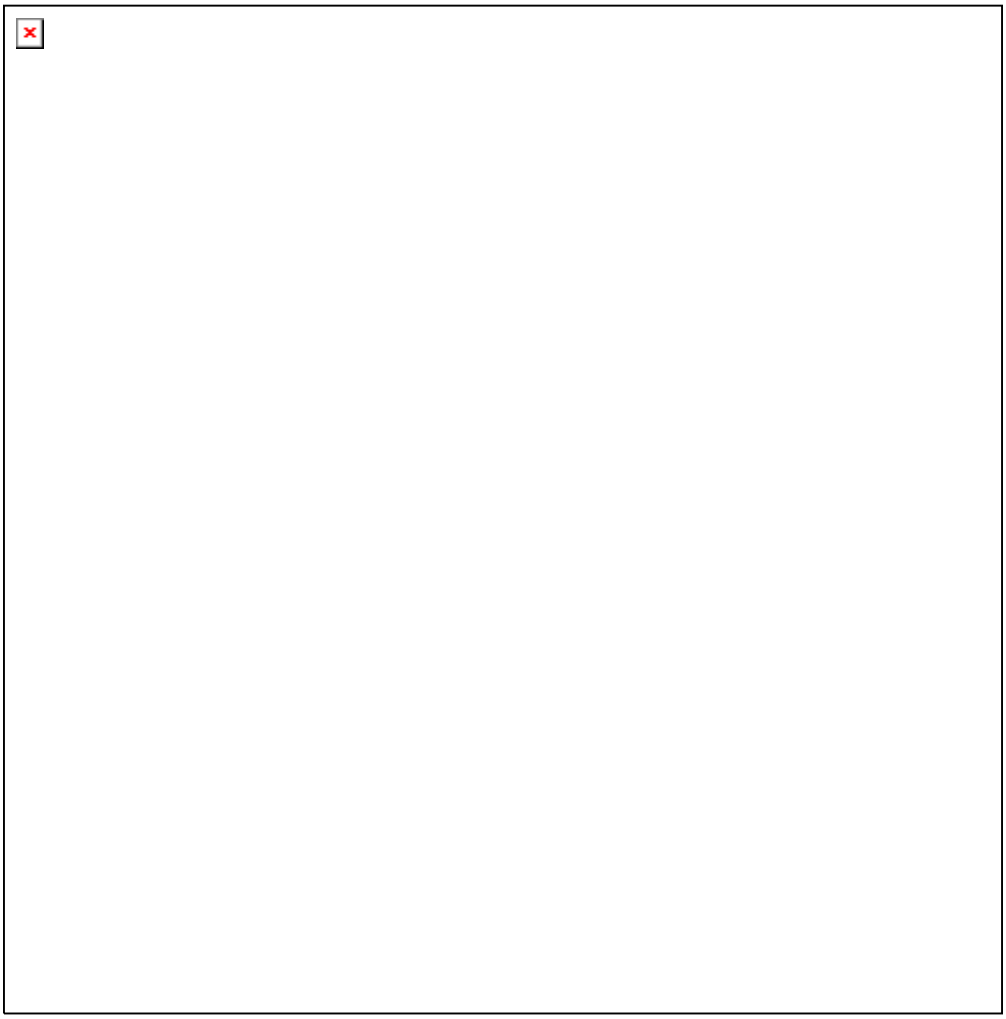

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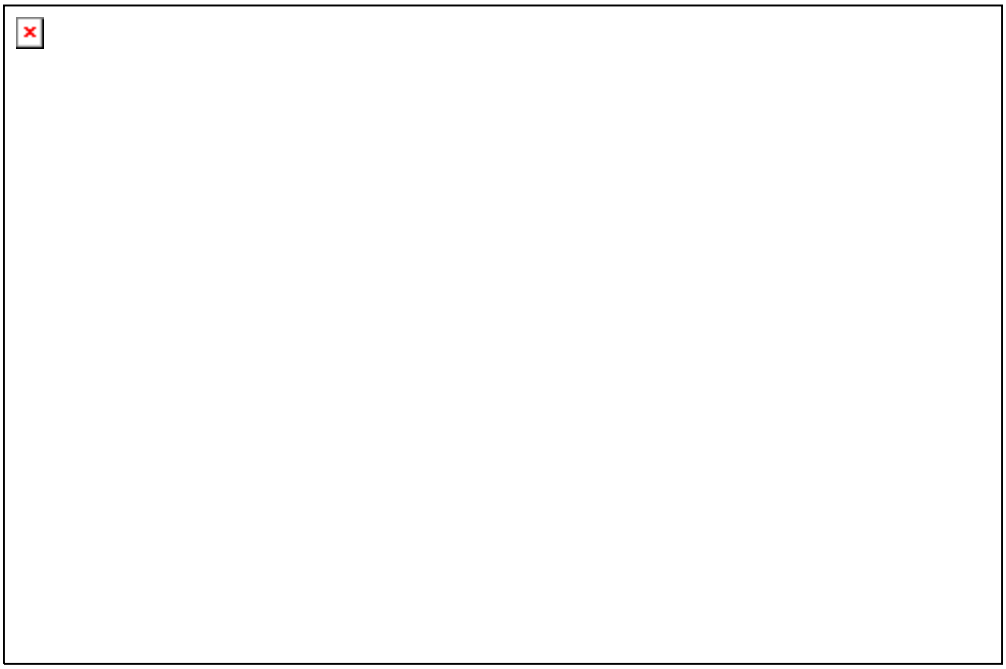

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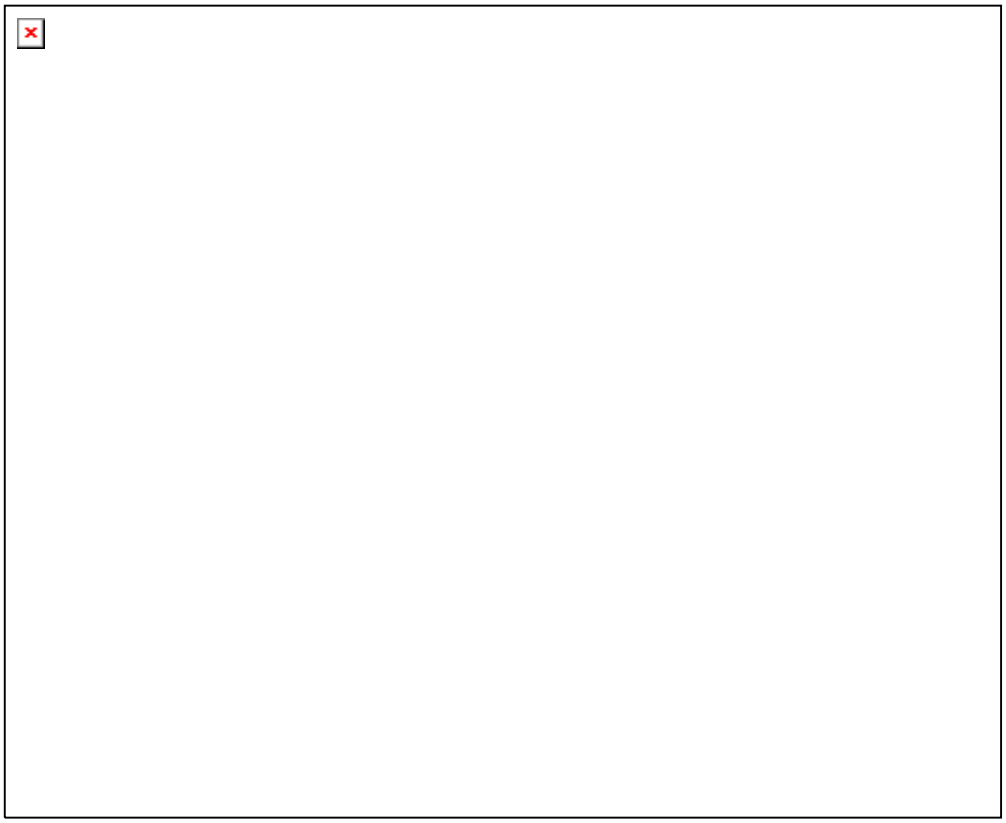

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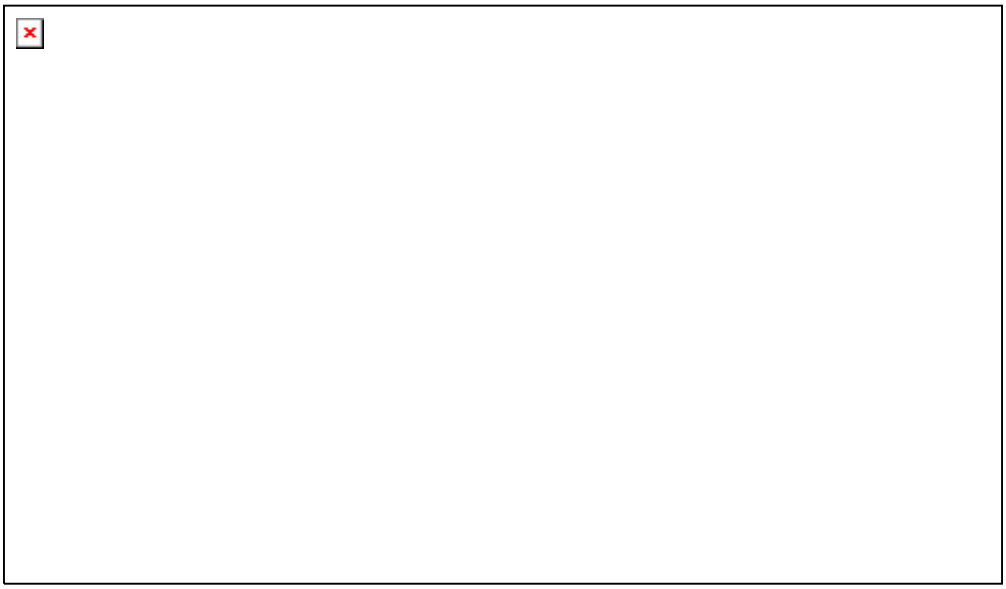

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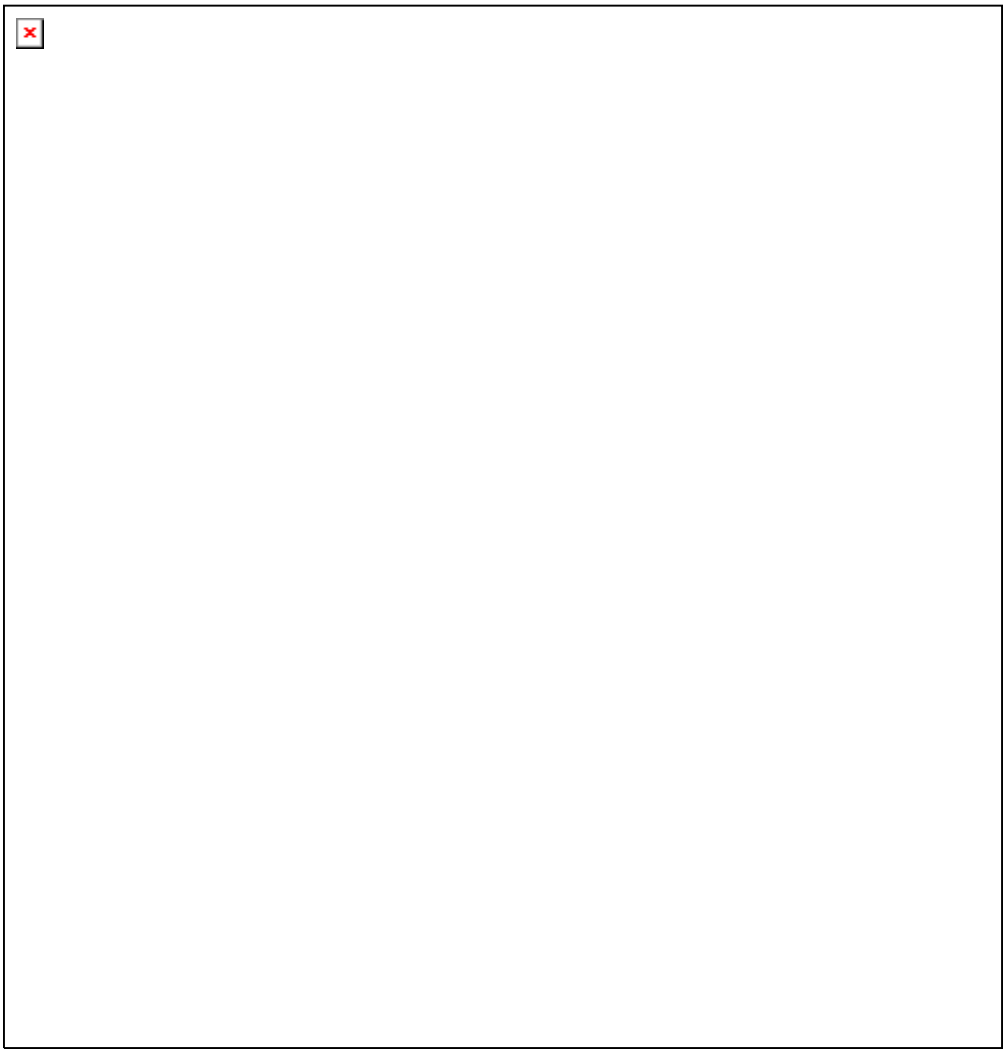

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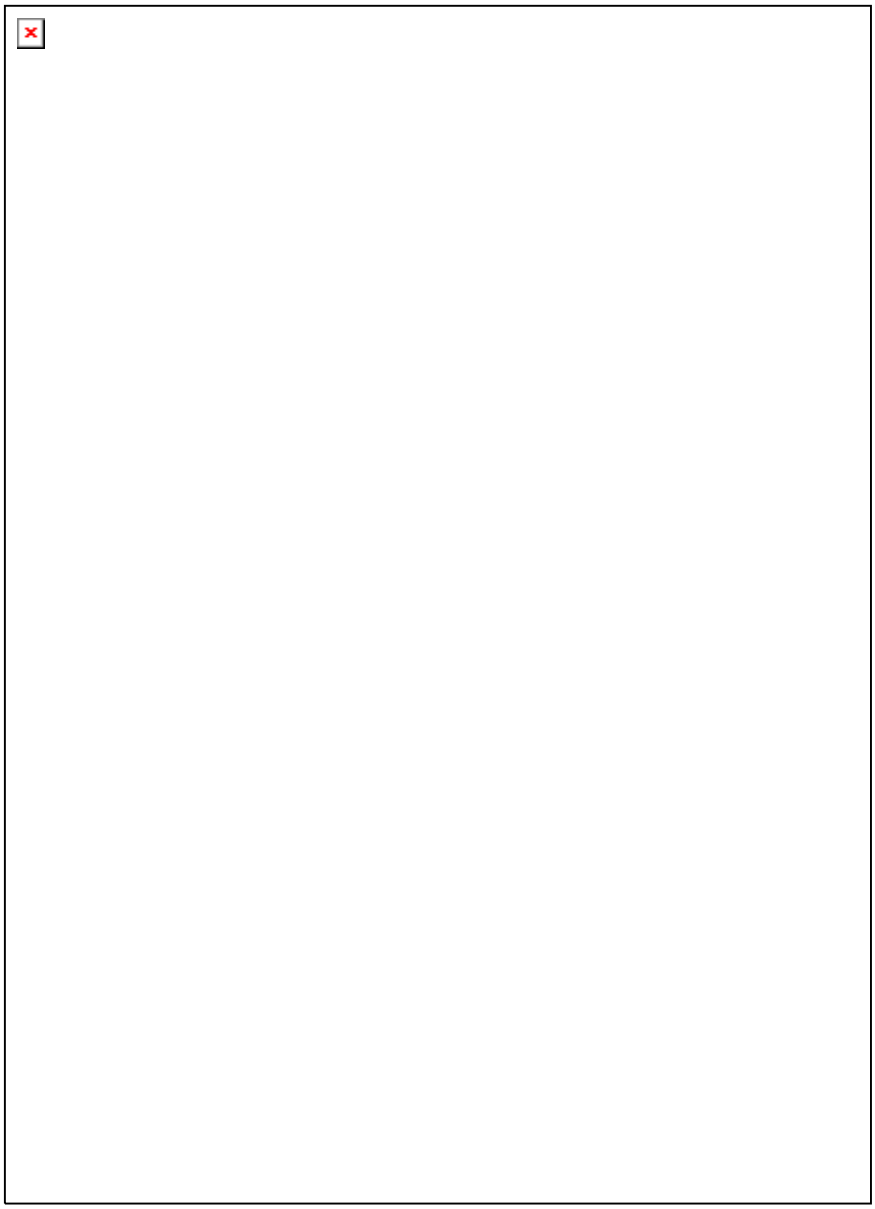

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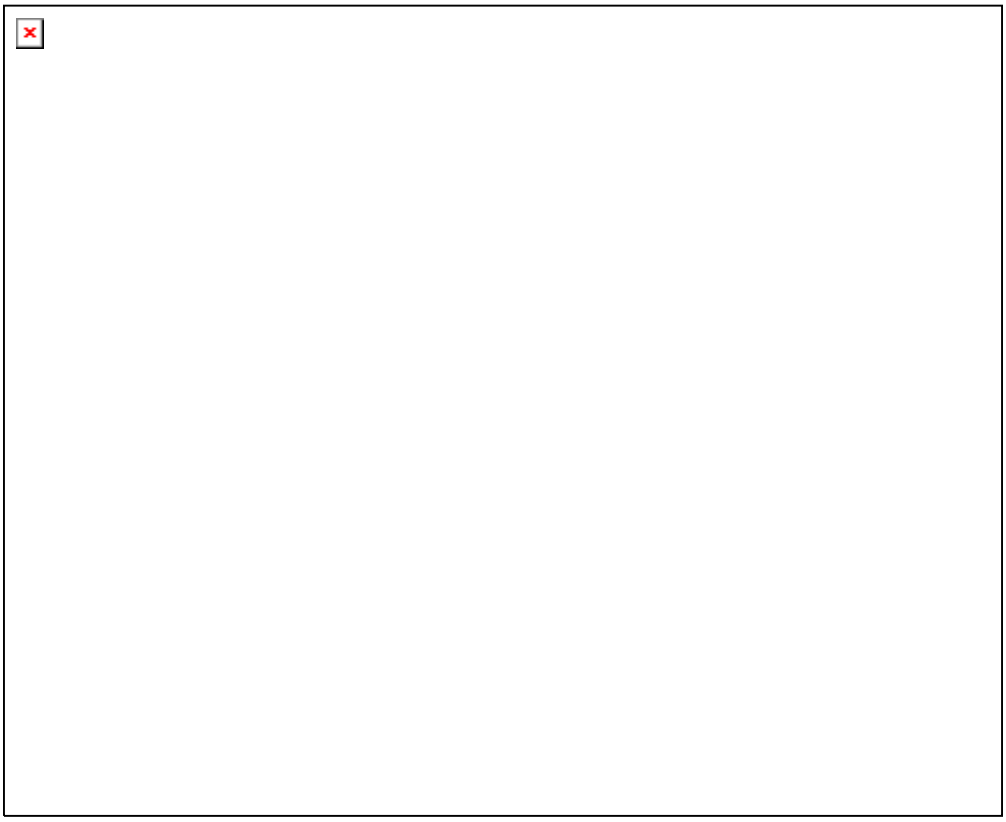

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