The root zone of graminoids: A niche for H₂-consuming acetogens in a minerotrophic peatland

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The importance of acetogens for H₂ turnover and overall anaerobic degradation in peatlands remains elusive. In the well-studied minerotrophic peatland fen Schlöppnerbrunnen, H₂-consuming acetogens are conceptualized to be largely outcompeted by iron reducers, sulfate reducers, and hydrogenotrophic methanogens in bulk peat soil. However, in root zones of graminoids, fermenters thriving on rhizodeposits and root litter might temporarily provide sufficient H₂ for acetogens. In the present study, root-free peat soils from around the roots of Molinia caerulea and Carex rostrata (i.e., two graminoids common in fen Schlöppnerbrunnen) were anoxically incubated with or without supplemental H₂ to simulate conditions of high and low H₂ availability in the fen. In unsupplemented soil treatments, H₂ concentrations were largely below the detection limit (∼10 ppmV) and possibly too low for acetogens and methanogens, an assumption supported by the finding that neither acetate nor methane substantially accumulated. In the presence of supplemental H₂, acetate accumulation exceeded CH₄ accumulation in Molinia soil whereas acetate and methane accumulated equally in Carex soil. However, reductant recoveries indicated that initially, additional unknown processes were involved either in H₂ consumption or the consumption of acetate produced by H₂-consuming acetogens. 16S rRNA and 16S rRNA gene analyses revealed that potential acetogens (Clostridium, Holophagaceae), methanogens (Methanocellales, Methanobacterium), iron reducers (Geobacter), and physiologically uncharacterized phylotypes (Acidobacteria, Actinobacteria, Bacteroidetes) were stimulated by supplemental H₂ in soil treatments. Phylotypes closely related to clostridial acetogens were also active in soil-free Molinia and Carex root treatments with or without supplemental H₂. Due to pronounced fermentation activities, H₂ consumption was less obvious in root treatments, and acetogens likely thrived on root organic carbon and fermentation products (e.g., ethanol) in addition to H₂. Collectively, the data highlighted that in fen Schlöppnerbrunnen, acetogens are associated to graminoid roots and inhabit the peat soil around the roots, where they have to compete for H₂ with methanogens and iron reducers. Furthermore,
Introduction

Acetogens are a polyphyletic group of anaerobes that can use the reductive acetyl-CoA pathway for dissimilation (Drake et al., 2008; Ragsdale, 2008). During hydrogenotrophic acetogenesis $H_2$ is used as electron donor ($4H_2 + CO_2 \rightarrow CH_3COO^- + H^+$) whereas organic electron donors (e.g., glucose [$C_6H_12O_6 \rightarrow 3CH_3COO^- + 3H^+$] or ethanol [$2CH_3CH_2OH + 2CO_2 \rightarrow 3CH_3COO^- + 3H^+$]) are used during organotrophic acetogenesis (Lever, 2012; Schuchmann and Müller, 2014, 2016). Considering their metabolic versatility, it is not surprising that acetogens were isolated from various ecosystems including peatlands (Kotsyurbenko et al., 1995; Simankova et al., 2000; Drake et al., 2006; Göflner et al., 2008). Peatlands are waterlogged soil ecosystems that are of global relevance, especially because of their function as sinks for $CO_2$ and sources for $CH_4$ (Aselmann and Crutzen, 1989; Yu et al., 2010; Abdalla et al., 2016; Harenda et al., 2018). Despite recent findings showing that acetogens contribute to the production of acetate in some peatlands, their ecological significance in these ecosystems is still conceptualized rather than resolved (Drake et al., 2009; Hunger et al., 2011; Hädrich et al., 2012; Ye et al., 2014; Kotsyurbenko et al., 2019).

The marked accumulation of acetate and increasing relative abundances of acetogenic taxa in peat soil incubations with supplemental $H_2$ suggested that acetogens can successfully compete for $H_2$ when it is available at sufficiently high concentrations in peatlands (Kotsyurbenko et al., 1996; Bräuer et al., 2004; Wüst et al., 2009; Hädrich et al., 2012; Hunger et al., 2015). However, at the low $H_2$ concentrations that are characteristic for bulk peat soil, acetogens are conceptualized to be outcompeted by methanogens and other $H_2$ consumers with lower $H_2$ thresholds (Drake et al., 2009; Estop-Aragonés et al., 2013; Kotsyurbenko et al., 2019). The competitiveness of acetogens increases at low temperatures predominating in northern peatlands (Conrad and Wetter, 1990; Nozhevnikova et al., 1994; Kotsyurbenko et al., 2001; Metje and Frenzel, 2005, 2007; Lever, 2012). Furthermore, it was suggested that acetogens may thrive in microenvironments within the peat soil in which $H_2$ concentrations might be higher than in the surrounding bulk peat (Hädrich et al., 2012; Ye et al., 2014; Schmidt et al., 2016). The study underscored that the metabolically flexible acetogens do not rely on $H_2$, potentially a key advantage over other $H_2$ consumers under the highly dynamic conditions characteristic for the root-zones of graminoids in peatlands.

Materials and methods

Sampling site and setup of anoxic incubations

Fen Schlöppnerbrunnen is a moderately acidic (pH 4.3–5.6), minerotrophic, $CH_4$-emitting fen that is completely overgrown with $M. caerulea$, intermingled with patches of sedges (e.g.,...
C. rostrata), rushes, and mosses; the fen is located in the Lestenbach catchment in the Fichtelgebirge (50°07′53″N and 11°52′51″E), Germany (Hamberger et al., 2008; Reiche et al., 2009; Hadrich et al., 2012).

The sampling of roots and soil as well as the setup of anoxic incubations largely resembled that of previous studies (Hunger et al., 2016; Meier et al., 2021) and is summarized in Supplementary Figure 1. Carex roots and Carex soil (i.e., peat soil from around the Carex roots) were sampled in July 2016; Molinia roots and Molinia soil (i.e., peat soil from around the Molinia roots) were sampled in July 2018. Samples were transported to the lab in airtight sterile plastic bags on ice and transferred in an anoxic chamber (100% N₂ atmosphere, Mecaplex, Grenchen, Switzerland). Roots were separated from the soil and washed gently with sterile anoxic water to remove residual soil particles; soil was sieved to obtain soil largely devoid of roots, termed root-free soil. One gram fresh weight of roots or soil were transferred in 27 ml glass tubes and 9 ml of anoxic mineral solution (Hunger et al., 2015) were added to make up a total volume of 10 ml. Tubes were sealed with butyl-rubber stoppers and flushed with 100% N₂. Approximately 10 µmol H₂ per ml liquid volume was added to H₂ treatments, whereas no H₂ was added to unsupplemented treatments. The following abbreviations are used for H₂ treatments and unsupplemented treatments: SUC, unsupplemented Carex roots; SHC, H₂ supplemented Carex roots; SUM, unsupplemented Molinia soil; SHM, H₂ supplemented Molinia soil; RUC, unsupplemented Carex roots; RHC, H₂ supplemented Carex roots; RUM, unsupplemented Molinia roots; and RHM, H₂ supplemented Molinia roots. All treatments were setup in triplicates and incubated in the dark at 15°C for 17 days (without a pre-incubation).

Chemical analyses

The headspaces and liquid phases of H₂ treatments and unsupplemented treatments were sampled regularly during the incubation using sterile syringes. The devices and instrumental setup used for (a) gas chromatographic analysis of headspace gasses, (b) high performance liquid chromatography analysis of organic acids and ethanol, and (c) pH measurements were those recently described in detail (Meier et al., 2021). Amounts of CO₂ (including pH-dependent amounts of bicarbonate), H₂, and CH₄ in the headspaces and liquid phases were calculated as described before (Meier et al., 2021), and molar concentrations of gasses were calculated by dividing total amounts of a gas (in µmol) by 9.5 ml (the volume of the liquid phase after initial sampling). Dry weight contents (determined by weighing before and after drying at 60°C for 72 h) of the roots and soil in the three experiments were as follows: Carex soil/roots, 11%/13%; Molinia soil/roots, 16%/36%. Millimolar concentrations of gasses, organic acids, and ethanol can be converted to µmol per g dry weight by multiplying with 86/73 for Carex soil/roots and 59/26 for Molinia soil/roots.

Reductant recoveries and thermodynamic calculations

Reductant recoveries were calculated to determine whether the enhanced accumulation of acetate and methane in H₂ supplemented soil treatments compared to unsupplemented soil treatments could be explained by the consumption of exogenous H₂. Reductant recoveries for acetate (Rₐ) and CH₄ (Rₘ) were calculated according to the following Equations.

\[
R_\text{a} = \frac{8 \times (\{[\text{H}_2\text{I}_2] - [\text{H}_2\text{I}_1]\} - \{[\text{Au}_2] - [\text{Au}_1]\})}{2 \times ([\text{Hh}_1] - [\text{Hh}_0]) - ([\text{Hu}_2] - [\text{Hu}_1])} \times 100% \\
R_\text{m} = \frac{8 \times ([\text{Mh}_2] - [\text{Mh}_1]) - ([\text{Mu}_2] - [\text{Mu}_1])}{2 \times ([\text{Hh}_1] - [\text{Hh}_0]) - ([\text{Hu}_2] - [\text{Hu}_1])} \times 100%
\]

In the equations above, eight refers to the number of reducing equivalents per molecule acetate and methane; two refers to the reducing equivalents per molecule H₂. [Ah], [Au], [Mh], [Mu], [Hh], and [Hu] are the concentrations of acetate in H₂ supplemented soil treatments, acetate in unsupplemented soil treatments, CH₄ in H₂ supplemented soil treatments, CH₄ in unsupplemented soil treatments, H₂ in H₂ supplemented soil treatments, and H₂ in unsupplemented soil treatments, respectively, at the start (t₁) and the end (t₂) of the respective time frame.

Gibbs free energies (ΔG) were calculated using the Nernst and Van’t Hoff equations (Conrad and Wetter, 1990).

Extraction of nucleic acids and reverse transcription of RNA

Three replicate nucleic acid extractions were performed with fresh washed roots and sieved soil to analyze the in situ microbial community at the time of sampling. At the end of the 17-day incubation, nucleic acids were extracted from all replicates of root and soil treatments separately. Samples of soil treatments were centrifuged at 16,000 × g for 15 min at 4°C (1-15-K Sartorius, Göttingen, Germany) to retrieve pelleted soil suitable for nucleic acid extraction. Nucleic acid extraction, digestion of DNA (to retrieve pure RNA) or RNA (to retrieve pure DNA), and cDNA synthesis were performed as described before (Meier et al., 2021).

Molecular analyses

PCR amplification, Illumina MiSeq amplicon sequencing, and data processing were performed as stated elsewhere.
Identification of phylotypes stimulated by supplemental H$_2$ in soil treatments

Linear discriminant analysis effect size (LEfSe; Segata et al., 2011) was conducted with 16S rRNA and 16S rRNA gene sequence data separately for Carex and Molinia soil treatments. Phylotypes that fulfilled the following three criteria based on sequence data separately for Carex and Molinia 2011) was conducted with 16S rRNA and 16S rRNA gene sequence data designated as “stimulated” by supplemental H$_2$: Phylotypes (a) were significantly ($P \leq 0.05$; Kruskal–Wallis test) more abundant in H$_2$ supplemented soil treatments compared to unsupplemented soil treatments, (b) had an effect size (LDA score) of $\geq 3$, and (c) were on average at least twice as abundant in H$_2$ supplemented soil treatments as in unsupplemented soil treatments (the proof of criteria c is not implemented in LEfSe and was performed manually in order to eliminate phylotypes that were only slightly more abundant in H$_2$ supplemented soil treatments compared to unsupplemented soil treatments).

Statistical analyses

One-sided Wilcoxon rank sum test implemented in R$^2$ was used to identify statistically significant differences ($P$ value of $\leq 0.05$) between the amounts of CH$_4$ and acetate formed in H$_2$ treatments and unsupplemented treatments during the 17-day incubation. Differences in the overall composition of prokaryotic communities before incubation and after incubation in H$_2$ treatments and unsupplemented treatments were visualized by non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance matrix calculated using the software Past3$^2$ (Hammer et al., 2001).

Accession numbers

Sequences were deposited at the European Nucleotide Archive under study numbers PRJEB37304 and PRJEB37863 for Carex and Molinia experiments, respectively. Representative sequences of phylotypes stimulated by supplemental H$_2$ in soil treatments were deposited under the accession numbers LR792771-LR792783 and LR792811-LR792818.

Results and discussion

Response to H$_2$ in soil treatments

In the fen Schlöppnerbrunnen, excess H$_2$ formed by root associated fermenters might occasionally diffuse into the peat soil surrounding graminoid roots where it might stimulate H$_2$ consumers that rely on H$_2$ concentrations higher than the 0.2–28 nmol l$^{-1}$ dissolved H$_2$ (corresponds to a H$_2$ partial pressure of approximately 0.03–4 Pa) observed in bulk peat soil in situ (Knorr et al., 2009; Estop-Aragonés et al., 2013; Hunger et al., 2016; Meier et al., 2021). To simulate contrasting H$_2$ availabilities in the peat soil, soil treatments with supplemental H$_2$ and unsupplemented soil treatments were conducted.

Supplemental H$_2$ was consumed linearly in H$_2$ supplemented soil treatments at rates of 0.45 mM H$_2$ d$^{-1}$ ($R^2 = 0.98$) for Carex soil (Treatment SHC) and 0.48 mM H$_2$ d$^{-1}$ ($R^2 = 0.97$) for Molinia soil (Treatment SHM; Figure 1A). That H$_2$ was consumed without delay suggested that H$_2$-consuming microbes in the root zones of both plants were poised to respond quickly to the sudden availability of H$_2$ at high concentrations. Initially, the consumption of H$_2$ was in stark contrast to the accumulation of little acetate and CH$_4$ in treatments SHC and SHM (Figure 1A), and redundant recoveries confirmed that both products accounted for only a small fraction of the H$_2$ that was consumed within the first 6 to 7 days (Table 1). Thus, neither hydrogenotrophic acetogenesis nor hydrogenotrophic methanogenesis seemed to be main H$_2$-consuming processes during the first stage of incubation. With time acetate and CH$_4$ accumulation accelerated (Figure 1A), and during the second stage of incubation, both products collectively accounted for 87.4 and 89.6% of consumed exogenous H$_2$ in Treatments SHC and SHM, respectively, (Table 1), pointing toward hydrogenotrophic acetogenesis and hydrogenotrophic methanogenesis as the main H$_2$-consuming processes between day 6 or 7 and day 17. CO$_2$ is the electron acceptor of both processes (Thauer et al., 2008; Schuchmann and Müller, 2014), and its subsequent consumption toward the end of the incubation is in line with ongoing hydrogenotrophic acetogenesis and hydrogenotrophic methanogenesis in Treatments SHC and SHM (Figure 1A). Similar amounts of CH$_4$ and acetate were formed in Treatment SHC, whereas at least three times more acetate than CH$_4$ accumulated in Treatment SHM (Figure 1A). Small amounts of propionate accumulated toward the end of the incubation in H$_2$ supplemented soil treatments (Supplementary Figure 2), a finding in line with the formation of propionate in formate treatments of fen Schlöppnerbrunnen soil (Hunger et al., 2011). In unsupplemented Molinia soil (Treatment SUM) low
FIGURE 1
Concentrations of H$_2$, CO$_2$, CH$_4$, and acetate in soil treatments (A) and root treatments (B). Treatment identifiers: SUC, unsupplemented Carex soil; SHC, H$_2$ supplemented Carex soil; SUM, unsupplemented Molinia soil; SHM, H$_2$ supplemented Molinia soil; RUC, unsupplemented Carex roots; RHC, H$_2$ supplemented Carex roots; RUM, unsupplemented Molinia roots; RHM, H$_2$ supplemented Molinia roots. Symbols: circles, replicate 1; triangles, replicate 2; squares, replicate 3. The asterisks indicate significant differences (one-sided Wilcoxon rank sum test; $P \leq 0.05$) between the amounts of CH$_4$ and acetate formed in H$_2$ treatments and unsupplemented treatments during incubation. See Supplementary Figure 2 for ethanol, butyrate, propionate, and pH.

amounts of acetate and CH$_4$ accumulated toward the end of the incubation, and in unsupplemented Carex soil (Treatment SUC) neither acetate nor CH$_4$ accumulated (Figure 1A).

The collective data suggested that H$_2$ consuming acetogens and methanogens inhabiting the peat soil surrounding Carex and Molinia roots were substrate limited in unsupplemented
soil treatments and became increasingly important in H₂ supplemented soil treatments. While acetogenic and methanogenic potentials were similar in H₂ treatments with Carex soil, acetogenesis was found to exceed methanogenesis in H₂ treatments with Molinia soil. In any way, anaerobic respiratory microbes that use electron acceptors others than CO₂ were presumably involved in the mineralization of peat organic carbon and in the consumption of supplemental H₂. It cannot be excluded that such respiratory processes also consumed acetate produced by acetogens, and aceticlastic methanogenesis is another possible sink for acetate.

### Phylotypes stimulated by supplemental H₂ in soil treatments

Non-metric multidimensional scaling analyses of 16S rRNA and 16S rRNA gene phylotypes (≥97% sequence similarity), alpha diversity parameters, and phylum/family level-based community profiling collectively suggested that supplemental H₂ had a minor effect on the overall microbial community composition in soil treatments and root treatments of both plants (Supplemental Text 1, Supplementary Figures 3, 4, and Supplementary Tables 1, 2). Thus, a more detailed analysis was necessary to identify potential soil-born or root-associated H₂ consumers.

In order to identify the most important 16S rRNA phylotypes that responded to supplemental H₂ in soil treatments a two-step approach was conducted (see section “Material and methods” for details). LEfSe analyses (Segata et al., 2011) identified 17 Carex phylotypes and 10 Molinia phylotypes that (1) were significantly more abundant in H₂ supplemented soil treatments than in unsupplemented soil treatments and (2) had LDA-scores of three or higher (Table 2). 13 of the 17 Carex phylotypes and 6 of the 10 Molinia phylotypes were at least twice as abundant in H₂ supplemented soil treatments than in unsupplemented soil treatments and only these phylotypes were considered as "stimulated by H₂" (Table 2). Phylogenetic analysis revealed that 6 of the 13 Carex phylotypes shared 100% 16S rRNA gene sequence similarity with Molinia phylotypes that fulfilled the LEfSe criteria; hereafter, these phylotypes were designated as "shared phylotypes" (S, e.g., phylotype S1 comprises Carex phylotype C50 and Molinia phylotype M7; Figure 2).

The phylotypes stimulated by H₂ collectively accounted for 7.9–13.4% of the 16S rRNA sequences and 3.8–5.7% of the 16S rRNA gene sequences in Treatment SHC or SHM, which was higher than in unsupplemented soil (Treatments SUC and SUM) and fresh soil (SFC and SFM; Figure 3). Some of these phylotypes were phylogenetically affiliated with aceticogenic Clostridium species, methanogenic Euryarchaeota, and iron reducers of the genus Geobacter. Other phylotypes fell within the physiologically diverse phylum Acidobacteria or were only distantly related to any cultured organism (Figure 2).

In Table 3, potential ecological functions of the phylotypes stimulated by H₂ were discussed based on physiological traits and genomic potentials of cultured relatives.

In summary, the manually refined LEfSe approach conducted in this study was appropriate to reveal phylotypes stimulated by H₂ in soil treatments. Some of these phylotypes were related either to hydrogenotrophic acetogens or hydrogenotrophic methanogens and these phylotypes might have contributed to the observed accumulation of acetate and CH₄, respectively, (Figure 1A). Other phylotypes presumably represent iron reducers and might have been involved in early consumption of exogenous H₂ (Table 1); alternatively, they were stimulated by acetate derived from hydrogenotrophic acetogenesis.

### Anaerobic processes driven by peat organic carbon in soil treatments

H₂ partial pressures in unsupplemented soil treatments were mostly below the detection limit of ~10 ppmV. Hence, the calculation of Gibbs free energies (∆Gs) for hydrogenotrophic methanogenesis is another possible sink for acetate.

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**Table 1** Reductant recoveries for supplemental H₂ consumed in soil H₂ treatments.

| Treatment | Incubation period [d] | Consumed H₂ [mM] | % of reductant recovered in a product |
|-----------|-----------------------|------------------|--------------------------------------|
|           |                       |                  | CH₄ | Acetate | Unknown |
| SHC       | 0–6                   | 3.6              | 2.5 | 0.9    | 98.4    |
|           | 6–17                  | 4.4              | 46.3| 41.1   | 12.6    |
|           | 0–17                  | 8.0              | 26.5| 22.1   | 51.3    |
| SHM       | 0–7                   | 4.5              | 0.6 | 12.0   | 87.4    |
|           | 7–17                  | 3.6              | 20.6| 68.9   | 10.4    |
|           | 0–17                  | 8.1              | 9.5 | 37.3   | 53.2    |

*Recoveries were calculated from mean (n = 3) concentrations of H₂, CH₄, and acetate of H₂ treatments and unsupplemented treatments (Figure 1A) as described in the material and methods section. Percentages of reductant recovered in unknown products (e.g., sulfide, ferrous iron, and reduced humic acids) were calculated by subtracting percentages of reductant recovered in CH₄ and acetate from 100%.

1Treatment: SHC, H₂ supplemented Carex soil; SHM, H₂ supplemented Molinia soil.

2The negative value indicates that initially acetate accumulation was lower in H₂ supplemented Carex soil compared to unsupplemented Carex soil.
TABLE 2 Phylotypes stimulated by supplemental H\textsubscript{2} in soil treatments a.

| PT (S)\textsuperscript{b} | LDA-Score\textsuperscript{c} | RA ratio |
|--------------------------|-----------------------------|----------|
|                          | 16S rRNA\textsuperscript{d} | 16S rRNA genes\textsuperscript{d} | 16S rRNA\textsuperscript{d} | 16S rRNA genes\textsuperscript{d} |
| \textbf{Carex phylotypes} |               |                      |                   |                       |
| C50 (1)                  | 3.86\textsuperscript{e}  | 3.67\textsuperscript{f}  | 21.7              | 44.3                   |
| C157 (2)                 | 3.67\textsuperscript{g}  | 3.36\textsuperscript{h}  | 15.7              | 6.4                    |
| C67 (3)                  | 3.99\textsuperscript{i}  | 3.21\textsuperscript{j}  | 49.6              | 3.4                    |
| C81 (4)                  | 3.27\textsuperscript{k}  | 3.49\textsuperscript{l}  | 8.8               | 3.2                    |
| C207 (5)                 | 3.13\textsuperscript{m}  | 3.12\textsuperscript{n}  | 14.6              | 13                     |
| C85 (6)                  | 3.65\textsuperscript{o}  | 3.15\textsuperscript{p}  | 2.4               | 2.2                    |
| C148                     | 3.47\textsuperscript{q}  | –                      | 16.7              | 10.3                   |
| C980                     | –                          | 3.12\textsuperscript{r}  | 13                | 5.8                    |
| C186                     | n.a.\textsuperscript{s}   | 3.18\textsuperscript{t}  | n.a.\textsuperscript{s} | 345.4                 |
| C198                     | 3.22\textsuperscript{u}  | 3.10\textsuperscript{v}  | 63.7              | 18.4                   |
| C200                     | 3.31\textsuperscript{w}  | 3.14\textsuperscript{x}  | 5.8               | 3.4                    |
| C21                      | 3.09\textsuperscript{y}  | –                      | 2.3               | 0.9                    |
| C2605                    | 3.29\textsuperscript{z}  | –                      | 162.7             | 172.8                  |
| C15                      | 3.76\textsuperscript{|}  | –                      | 1.3               | 1.0                    |
| C43                      | 3.49\textsuperscript{~}  | –                      | 1.8               | 0.8                    |
| C58                      | 3.15\textsuperscript{a}  | –                      | 1.4               | 1.1                    |
| C2133                    | 3.39\textsuperscript{b}  | –                      | 1.7               | 0.9                    |
| \textbf{Molinia phylotypes} |               |                      |                   |                       |
| M7 (1)                   | 3.18\textsuperscript{c}  | 3.37\textsuperscript{d}  | 2.6               | 4.9                    |
| M44 (2)                  | 3.63\textsuperscript{e}  | 3.37\textsuperscript{f}  | 1.9               | 1.5                    |
| M150 (3)                 | 3.27\textsuperscript{g}  | –                      | 7.4               | 4.2                    |
| M21 (4)                  | 3.73\textsuperscript{h}  | –                      | 120.0             | 7.5                    |
| M77 (5)                  | 3.59\textsuperscript{i}  | 3.15\textsuperscript{j}  | 2.5               | 2.1                    |
| M39 (6)                  | 3.78\textsuperscript{k}  | –                      | 1.6               | 1.6                    |
| M78                      | 3.80\textsuperscript{l}  | –                      | 51.0              | 84.3                   |
| M106                     | 3.94\textsuperscript{m}  | 3.35\textsuperscript{n}  | 3.5               | 3.6                    |
| M33                      | 3.19\textsuperscript{o}  | –                      | 1.7               | 1.7                    |
| M55                      | –                          | 3.14\textsuperscript{p}  | 1.0               | 1.2                    |

\textsuperscript{a}Listed are phylotypes that fulfilled the following criteria of LEfSe analyses (Segata et al., 2011): significantly (P ≤ 0.05, Kruskal–Wallis test) higher relative abundances in H\textsubscript{2} supplemented soil treatments compared to unsupplemented soil treatments and effect sizes (LDA-scores) of ≥3. Phylotypes printed in italics had relative abundance ratios (RA ratios; calculated by dividing mean relative abundances of H\textsubscript{2} supplemented soil treatments (SHC and SHM) by those of unsupplemented soil treatments (SUC and SUM)) of ≥2; these phylotypes were considered as “stimulated by H\textsubscript{2}.”

\textsuperscript{b}PT, phylotype; S, shared phylotypes (i.e., Carex phylotypes that shared 100% 16S rRNA gene sequence similarity with Molinia phylotypes; see Figure 2).

\textsuperscript{c}Numbers in parentheses display the rank in the LEfSe-Linear discriminant analyses. –, the phylotype had a LDA score of <3 or had a P value of >0.05 in the Kruskal–Wallis test.

\textsuperscript{d}Analyses were based on relative abundances of 16S rRNA and 16S rRNA genes, respectively.

\textsuperscript{e}n.a., no 16S rRNA sequence of phylotype C186 was detected in Treatment SUC.

acetogenesis and hydrogenotrophic methanogenesis was not possible. However, solely CO\textsubscript{2} but neither acetate nor CH\textsubscript{4} accumulated in treatments SUC and SUM (Figure 1A), suggesting that, in the absence of root organic carbon, respiratory anaerobes thriving on alternative electron acceptors like ferric iron or sulfate were involved in the mineralization of organic carbon and outcompeted acetogens and methanogens for endogenous H\textsubscript{2}. This assumption is supported by long lag phases for methane accumulation and immediate iron reduction in unsupplemented bulk peat soil incubations from the study site (Reiche et al., 2008). Furthermore, thermodynamic calculations indicated that \textit{in situ} iron and sulfate reducers can outcompete acetogens and methanogens for dissolved H\textsubscript{2} in bulk peat soil of fenn Schlooppnerbrunnen (Knorr et al., 2009; Estop-Aragonés et al., 2013). Notably, 6–12 Pa H\textsubscript{2} were detected at day 17 in Treatment SUM and at this time hydrogenotrophic methanogenesis was sufficiently exergonic (Figure 4A) and CH\textsubscript{4} concentrations finally increased (Figure 1A). At the same time the mean ΔG for hydrogenotrophic acetogenesis was −5 kJ mol\textsuperscript{−1}, a value that has been shown to be the thermodynamic limit of the acetogen \textit{Acetobacterium carbinolicum} (Conrad and Wetter, 1990). In the aforementioned study \textit{A. carbinolicum} had H\textsubscript{2} thresholds of 10 Pa at 15°C (the incubation temperature in the present study). Thus, it cannot
FIGURE 2

16S rRNA gene–based phylogenetic trees of bacterial (A) or archaeal (B) phylotypes stimulated by supplemental H$_2$ in root-free soil treatments (bold; see Table 2) and related prokaryotes. S, shared phylotypes (i.e., Carex phylotypes that shared 100% 16S rRNA gene sequence similarity with Molinia phylotypes). The phylogenetic trees were calculated using the neighbor joining function (correction model: Jukes-Cantor) implemented in the ARB software (Ludwig et al., 2004). Bootstrap values (1,000 resamplings) higher than 70% are shown. Methanosarcina acetivorans C2A (AE010299) and Telmatobacter bradus TPB6017 (AM887760) were used as outgroup in (A,B), respectively.
be excluded that H$_2$-consuming acetogens contributed to the slight acetate accumulation observed in Treatment SUM at day 17 (Figure 1A).

**Consumers of supplemental H$_2$ in soil treatments**

In the initial incubation phase in H$_2$ supplemented soil treatments, consumption of exogenous H$_2$ was not coupled to the accumulation of acetate and CH$_4$ (Figure 1A and Table 1), pointing toward the activity of anaerobic H$_2$ consumers using electron acceptors others than CO$_2$. In this regard, consumption of exogenous H$_2$ (or formate) decoupled from methanogenesis and acetogenesis has been reported for soil incubations of fen Schlöppnerbrunnen before (Reiche et al., 2008; Hadrich et al., 2012; Hausmann et al., 2016). Nitrate, ferric iron, sulfate, and humic substances are alternative electron acceptors available in this fen (Estop-Aragonés et al., 2013). Since reported nitrate concentrations are low (0–150 µM) in the fen Schlöppnerbrunnen, it is unlikely that denitrification, a process that is proposed to be important in situ (Palmer et al., 2010, 2016), accounted for much of the consumed H$_2$ in soil treatments. In contrast, the cumulative
coupled to the reduction of ferric iron is supported by the
Knorr and Blodau, 2009; Knorr et al., 2009; Pester et al., 2012;
humic substances might suffice to account for the unresolved
in the incubated fen soil (Reiche et al., 2008; Bak et al., 1992; Liesack et al., 1994).

A possibility of Geobacter-affiliated PTs thriving on supplemental H2 and endogenous ferric iron in soil treatments. In addition, these PTs might have consumed acetate, an assumption supported by the ability of acetate oxidation reported for the reference organisms. PTs related to G. sp. FeAm09, G. bremensis and G. bemidjensis have been previously detected in enrichments of H2 or acetate utilizing iron reducers from another fen in the same catchment as the study site.

The stimulation of S4 by supplemental H2 was in line with the physiological properties of its closest cultured relative, Methanobacterium lacus AL-21 (99.7% Id.), a hydrogenotrophic methanogen isolated from a fen in Alaska. Methanobacterium sp. have been isolated from several peatlands thus far, but their importance in situ is unknown. Previous studies with fresh or incubated peat soil of the fen Schlöppnerbrunnen showed that relative abundances of Methanobacterium were lower than those of other hydrogenotrophic methanogens (e.g., Methanoregula and Methanocella).

S5, C980, C198, M106
S5 was closely related to Geobacter sp. FeAm09 (99.5% Id.), while G. bremensis and G. bemidjensis were the closest cultured relatives (97.7–97.9% Id.) of C980, C198, and M106. These reference organisms are metabolically versatile iron reducers that have been shown to utilize H2 (G. bremensis or G. bemidjensis) or have the genomic potential for H2 oxidation (Geobacter sp. FeAm09). Thus, it is possible that Geobacter-affiliated PTs thrived on supplemental H2 and endogenous ferric iron in soil treatments. In addition, these PTs might have consumed acetate, an assumption supported by the ability of acetate oxidation reported for the reference organisms. PTs related to G. sp. FeAm09, G. bremensis and G. bemidjensis have been previously detected in enrichments of H2 or acetate utilizing iron reducers from another fen in the same catchment as the study site.

S6
The closest cultured relative of S6 (95.3% Id.) was the peat acidobacterium Paludibaculum fermentans (subdivision 3 of the Acidobacteria), a sugar-utilizing facultative aerobe that can ferment and reduce ferric iron in the absence of O2 (its capability to utilize H2 was not tested). Considering the low identity, the physiological traits of S6 and P. fermentans may differ. Nevertheless, the pyrogenetic affiliation with an iron reducer and the finding that a phylotype closely related to S6 was detected in formate treatments of fen Schöppnerbrunnen soil, in which H2 levels were elevated, support the assumption that S6 may represent a H2-utilizing iron reducer. This assumption still needs verification.

C21 showed 100 % 16S rRNA sequence identity to Telmatobacter bradus (subdivision 1 of the Acidobacteria). T. bradus is a facultative aerobe that can ferment sugars and polysaccharides (including cellulose) to H2, CO2, acetate, and ethanol. Whether T. bradus can utilize H2 and reduce alternative electron acceptors like ferric iron or sulfate has not been tested yet. Based on metagenomic analyses it was proposed that some subdivision 1 Acidobacteria of the fen Schöppnerbrunnen might couple H2 oxidation to sulfide reduction. In addition, some isolates of subdivision 1 can reduce ferric iron. Nevertheless, it remains unresolved why the Telmatobacter-PT C21 was stimulated by H2 in the conducted soil treatments.

C186 and C148 were only distantly related (Id. <91%) to any cultured microbes. Closely related PTs have been detected in the fen before, but their physiologies remain unknown.

References: 1. Gäbler et al. (2008), 2. Bomar et al. (1991), 3. Liu et al. (2005), 4. Drake et al. (2008), 5. Bak et al. (1992), 6. Liesack et al. (1994), 7. Hunger et al. (2011), 8. Säkait et al. (2008), 9. Li and Lu (2013), 10. Gómez et al. (2003), 11. Hemberger et al. (2006), 12. Hunger et al. (2015), 13. Schmidt et al. (2015), 14. Kühn et al. (2016), 15. Cadillo-Quesada et al. (2015), 16. Zeller et al. (1982), 17. Kotlyarenko et al. (2005), 18. Steaub and Buchholz-Cleven (2001), 19. Nevins et al. (2016), 20. Kühn et al. (2016), 21. Yavas et al. (2018), 22. Kühn et al. (2018), 23. Kulichevskaya et al. (2014), 24. Pankratiev et al. (2012), 25. Hausmann et al. (2018), 26. Höhle et al. (2008), 27. PT, phylotype.
Gibbs free energies ($\Delta G$) of anaerobic processes in soil treatments (A) and root treatments (B). $\Delta G$s were calculated based on the following reactions: hydrogenotrophic acetogenesis, $4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$; hydrogenotrophic methanogenesis, $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$; aceticlastic methanogenesis, $CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$. $\bullet$, H$_2$ treatments; $\circ$, unsupplemented treatments; when H$_2$ was below the detection limit ($\sim 10$ ppmV) no $\Delta G$s could be calculated for hydrogenotrophic acetogenesis and hydrogenotrophic methanogenesis. Values represent means of triplicate analysis and error bars indicate standard deviations. Treatments: SHC, H$_2$ supplemented Carex soil; SUC, unsupplemented Carex soil; SHM, H$_2$ supplemented Molinia soil; SUM, unsupplemented Molinia soil; RHC, H$_2$ supplemented Carex roots; RUC, unsupplemented Carex roots; RHM, H$_2$ supplemented Molinia roots; and RUM, unsupplemented Molinia roots.

Subsequently, acetate and CH$_4$ accumulated in H$_2$ supplemented soil treatments, and reductant recoveries indicated that alternative electron acceptors might have been largely depleted and acetogens as well as methanogens were finally main H$_2$ consumers during this second incubation phase (Figure 1A and Table 1). That supplemental H$_2$ stimulated acetogenesis and methanogenesis in peat soil treatments corroborated previous studies suggesting that, in fen Schlöppnerbrunnen and other peatlands, acetogens and methanogens are limited by H$_2$ availability and both are poised to respond to temporarily higher H$_2$ concentrations (Kotsyurbenko et al., 1996; Bräuer et al., 2004; Wüst et al., 2009; Hunger et al., 2011, 2015, 2016; Hädrich et al., 2012). Four phylotypes that were stimulated by H$_2$ (Figure 3) were related to cultured acetogens (S1 to Clostridium drakei, C2605 to C. magnum, S2 and C200 to Holophaga foetida) and three to hydrogenotrophic methanogens (S3 and M78 to Methanolcella species and S4 to Methanobacterium lacus; Table 3). However, BLAST identities of Holophaga- and Methanolcella-related phylotypes to cultured relatives were “only” 95.1–96.6%; thus, isolation and physiological characterization are necessary to validate that these fen phylotypes indeed represent acetogens and methanogens.

**Potential acetate consuming processes in soil treatments**

Recently, high relative 16S rRNA abundances of Methanoseta and Methanosarcina in fresh peat soil from...
patches covered with Carex indicated that, locally, aceticlastic methanogens can make up a considerable fraction of the active prokaryotes in fen Schlöppnerbrunnen (Meier et al., 2021). In this regard, one phylotype (C44) was related to the aceticlastic methanogen Methanosarcina acetivorans (98.7% identity) and it accounted for 1.8–2.5% of all 16S rRNA sequences in the H₂ supplemented Carex soil (SHC, Supplementary Figure 4). Thus, it is possible that some of the acetate produced by acetogens thriving on supplemental H₂ was subsequently converted into CH₄ in Treatment SHC (Figure 1A). Likewise, acetate is a growth substrate of the iron reducing Geobacter species related to phylotypes S5, C980, C198, and M106 (Table 3), and one may speculate that these phylotypes were not exclusively thriving on supplemental H₂ but consumed acetate as well. This could explain why acetate accumulation was slow or absent at the beginning of incubation in treatments SHC and SHM, although hydrogenotrophic aceticlastic methanogenesis was sufficiently exergonic from the start (Figure 4A). Consequently, the calculated reductant recoveries for H₂ supplemented soil treatments likely underestimated the contribution of acetogens to the consumption of supplemental H₂ due to presumed acetate consumption by iron reducers and aceticlastic methanogens (Table 1). In situ, effective removal of acetate would be advantageous for acetogens since their capability to utilize low H₂ concentrations depends on low acetate concentrations (Schmidt et al., 2016).

Favorable thermodynamics for aceticlastic methanogenesis (< −60 kJ mol⁻¹; Figure 4A) and the finding that the Methanosarcina-related phylotype C44 accounted for 1.3–5.9% of the 16S RNA sequences in unsupplemented Carex soil (Treatment SUC, Supplementary Figure 4) raised the question why methane accumulation in this treatment was not observed. Efficient CH₄ removal via anaerobic CH₄ oxidation potentially coupled to the reduction of ferric iron is one possible explanation and was suggested to occur in peatlands before (Smemo and Yavitt, 2007, 2011; Yan et al., 2018).

### Anaerobic processes in root treatments

To evaluate H₂ consumption at contrasting H₂ availabilities by root-associated acetogens and methanogens, Carex roots and Molinia roots were anaerobically incubated with and without supplemental H₂. Product profiles of unsupplemented roots (Treatments RUC and RUM; Figure 1B and Supplementary Figure 2) corroborated recent findings that (1) H₂ is produced along with ethanol, CO₂, acetate, butyrate, and propionate during the fermentative degradation of root-derived organic carbon, and (2) the extent of H₂ accumulation varies between replicates (Meier et al., 2021). Toward the end of the 17-day incubation, H₂ consumption exceeded H₂ production in replicates RUC2 and RUM3, whereas H₂ production and consumption were balanced in RUC1 and RUC3, and H₂ production exceeded its consumption in RUM1 and RUM2 (Figure 1B). Independent of overall increasing or decreasing H₂ concentrations, it is likely that acetogens and fermenters were active at the same time. However, the experimental design did not allow to distinguish between acetate production by hydrogenotrophic acetogenesis, organotrophic acetogenesis, and fermentation.

Previously, formate-derived H₂, which was subsequently consumed, stimulated the production of acetate and methane in Carex root treatments (Hunger et al., 2016), and a similar stimulatory effect was anticipated for supplemental H₂ in the present study. However, H₂ concentrations were slowly decreasing or stayed constant in most replicates of root treatments with supplemental H₂, and neither the accumulation of acetate nor that of CH₄ (note that CH₄ was marginal in all root treatments) were significantly higher (P ≤ 0.05; Wilcoxon) in the H₂ supplemented roots (Treatments RHC and RHM) compared to unsupplemented roots (Treatments RUC and RUM; Figure 1B). Nevertheless, the strong accumulation of acetate paralleled by a decrease in H₂, as observed in RHC1 between day 10 and day 15 and in RHM3 between day 7 and day 11 (Figure 1B), were a strong indicator of hydrogenotrophic aceticlastic methanogenesis in these two replicates.

### Acetogenic phylotypes in root treatments

The manually refined LEiSe approach conducted to reveal phylotypes that were stimulated by supplemental H₂ in soil treatments was not appropriate for the identification of such phylotypes in root treatments (data not shown). In this regard, fermentation of root-derived organic carbon yielded presumably sufficient H₂ for root-associated H₂ consumers, largely obliterating the expected stimulative effect of supplemental H₂ in root treatments (Figure 1B). In an alternative approach, phylotypes with either ≥1% 16S rRNA or ≥0.5% 16S rRNA gene relative abundance in at least one replicate of any of the root treatments (RUC, RUM, RHC, and RHM) were subjected to a BLASTn analysis and the closest cultured relatives were manually screened for potential H₂ consumers (e.g., acetogens, methanogens, and iron reducers).

This analysis revealed one phylotype [S1; this phylotype was also active in soil treatments (Figure 3)] with 97.8% identity to the peat bog acetogen Clostridium drosaei FP (Göflner et al., 2008). In RHM3 and RHC1, i.e., the two replicates of root H₂ treatments in which H₂ consumption was most obvious (Figure 1B), phylotype S1 accounted for 9.4% and 3.8% of all 16S rRNA sequences, respectively, (Table 4). However, 16S rRNA and 16S rRNA gene relative abundances of S1 were high in Treatment RUM (Table 4), and in replicates of this treatment H₂ consumption was less obvious (Figure 1B). Thus, the relative
abundance of S1 did not in all cases correlate with observable H2 consumption.

A second potentially acetogenic phylotype [M227; 98.3% identity to the hydrogenotrophic acetogen Clostridium magnum (Bomar et al., 1991)] was observed in Molinia root treatments (Table 4). Collectively, S1 and M227, which were marginal in fresh Molinia root samples, accounted for up to 10.6% of the 16S rRNA sequences (in RHM3) and 8.8% of the 16S rRNA gene sequences (in RUM3), indicating that root-associated acetogens thrived in Molinia root treatments with and without supplemental H2 (Table 4).

Phylotypes S1 and M227 were 100% similar to G2 (acc. no. LR702023) and G1 (LR702022), respectively, and the latter two phylotypes were detected in unsupplemented Carex and Molinia root treatments before (Meier et al., 2021). Furthermore, S1 was 100% similar to an acetogen (LT009683; Figure 2) present in an acetogenic enrichment (FH) that was derived from a mixture of Carex and Molinia roots collected at fen Schloppnerbrunnen; enrichment FH converted formate and H2 to acetate (Hunger et al., 2016). That phylotypes related to C. drakei (S1) and C. magnum (M227) were repeatedly observed in treatments with Carex and Molinia roots suggests that clostridial acetogens colonize the roots of these graminoids in fen Schloppnerbrunnen.

### Acetogenesis and methanogenesis in root treatments

Thermodynamic calculations revealed that hydrogenotrophic acetogenesis was feasible in unsupplemented roots (Treatments RUC and RUM; Figure 4B) as soon as sufficient fermentation-derived H2 accumulated (after 3 to 6 days; Figure 1B). Furthermore, supplemental H2 did neither significantly stimulate acetate production (Figure 1B) nor were 16S rRNA and 16S rRNA gene relative abundances of potentially acetogenic phylotypes significantly higher in root treatments with supplemental H2 than in those without (Table 4). These findings indicated that acetogens in root treatments did not relay on supplemental H2. Nevertheless, H2 consumption in root treatments was most obvious in two replicates that received supplemental H2 (RHC1 and RHM3; Figure 1B). In RHM3 between day 7 and 11, concentrations of H2 and CO2 decreased by 6 mM and 2.8 mM, respectively, and acetate increased by 12.8 mM. However, only 1.5 mM acetate can be formed from 6 mM H2 via hydrogenotrophic acetogenesis. This discrepancy can be explained as follows: (1) A part of the accumulated acetate was presumably produced by fermenters thriving on root-derived organic carbon (Meier et al., 2021). (2) Only net consumption/production of H2, CO2, and acetate could be determined, but fermentative production of H2 and CO2 were most likely ongoing concomitantly to their consumption by acetogens in all root treatments. In this regard, radiotracer experiments indicated that 30–40% of acetate in rice root treatments originated from H2-CO2, although net consumption of H2 could only account for 4% of accumulated acetate, pointing toward a fast turnover of H2 in these rice root treatments (Conrad and Klose, 1999). (3) The metabolic versatility of acetogens is widely recognized (Drake et al., 2008), and, in the root treatments, acetogens might have utilized root-derived organic carbon (e.g., sugars) and fermentation products [e.g., formate, lactate, and ethanol (Weghoff et al., 2015; Bertsch et al., 2016; Hunger et al., 2016)] in addition to H2-CO2. In this respect, ethanol concentrations decreased by 4.8 mM in

### Table 4 Relative abundances of potentially acetogenic phylotypes in fresh and anoxically incubated roots a.  

| PT     | Sample or treatmentb | Relative abundance [%]c  |
|--------|----------------------|--------------------------|
|        |                      | 16S rRNA Rep 1 Rep 2 Rep 3 | 16S rRNA genes Rep 1 Rep 2 Rep 3 |
| S1     | RFC                  | 0.05 0.02 0.03           | 0.01 0.02 0.01 |
|        | RUC                  | 0.51 0.12 0.01           | 0.41 0.03 0.003 |
|        | RHC                  | 3.74 0.04 0.02           | 0.94 0.05 0.005 |
|        | RFM                  | 0.02 0.12 0.03           | 0.03 0.05 0.01 |
|        | RUM                  | 4.47 7.19 7.87           | 2.77 6.45 7.35 |
|        | RHM                  | 0.77 4.89 9.36           | 0.55 3.78 3.76 |
| M227   | RFC                  | 0.003 0.02 0.000         | 0.000 0.003 0.000 |
|        | RUM                  | 0.04 1.05 0.94           | 0.02 1.62 1.43 |
|        | RHM                  | 0.12 0.04 1.27           | 0.11 0.06 0.74 |

a Listed are Phylotypes (PT) with either \( \geq 1\% \) 16S rRNA or \( \geq 0.5\% \) 16S rRNA gene relative abundance in at least one replicate of any root treatment that were closely related to cultured acetogens: S1 with 97.8% identity to Clostridium drakei and M227 with 98.3% identity to Clostridium magnum.

b Identifiers: RFC, fresh Carex roots; RFM, fresh Molinia roots; RUC, unsupplemented Carex roots; RUM, unsupplemented Molinia roots; RHC, H2 supplemented Carex roots; and RHM, H2 supplemented Molinia roots.

cRep, replicates of fresh root samples or anoxic root treatments.
Implications for acetogens in the root-zone of fen graminoids

H₂ was an important product of fermenters thriving on decaying roots and root litter in Carex and Molinia root treatments conducted in this and previous studies (Hunger et al., 2016; Meier et al., 2021). In situ, exudates, constantly released by roots of photosynthetically active plants, are another important source of root-derived organic carbon (Jones et al., 2009), and they might be partially converted to H₂ as well. H₂ formed by root-associated fermenters can theoretically (1) be transported through the plants via the aerenchyma and emitted to the atmosphere, (2) be consumed by aerobic or anaerobic microbial H₂ consumers colonizing the roots, or (3) radially diffuse into the soil surrounding the roots where it is eventually consumed by soil microbes (Conrad, 1996). Acetogens inhabiting the root zone of graminoids could profit from locally and temporarily higher H₂ concentrations than the 0.2–28 nmol l⁻¹ dissolved H₂ (corresponds to a H₂ partial pressure of approximately 0.03–4 Pa) observed in bulk peat soil of the fen Schlöppnerbrunnen (Knorr et al., 2009; Estop-Aragonés et al., 2013). In fact, acetogens have been shown to be associated to the roots of several aquatic plants, and their ability to cope with oxic stress makes them less vulnerable to O₂ released from the roots than methanogens (Conrad and Klose, 1999; Küsel et al., 1999, 2001; Leaphart et al., 2003; Göflner et al., 2006). As discussed earlier (Conrad and Klose, 1999; Hunger et al., 2016; Meier et al., 2021), the conducted incubations cannot simulate the complex processes ongoing in the root zone of a living plant, but show the potential of its anaerobic microbial community to thrive on supplemental H₂, fermentation-derived H₂, and root or peat organic carbon.
In Figure 5 the interwoven trophic links in root and soil treatments were graphically summarized to help addressing the initial hypotheses: (1) Phyotypes related to cultured acetogens \((Clostridium\) and \(Holophaga\)) were identified in root treatments and soil treatments, underscoring that acetogens are associated to graminoid roots and inhabit the surrounding peat soil. However, these potential acetogens have yet to be cultured to validate their assumed physiology. (2) Acetogens most likely consumed fermentation derived \(\text{H}_2\) in root treatments and supplemental \(\text{H}_2\) in soil treatments, suggesting that \(\text{H}_2\) temporarily formed in excess at the immediate vicinity of graminoid roots, can be utilized by acetogens, if it accumulates to sufficiently high concentrations. In situ, \(\text{H}_2\) will likely diffuse away (Conrad, 1996) and not accumulate to concentrations as high as in root treatments or \(\text{H}_2\) supplemented soil treatments. Therefore, radial \(\text{H}_2\) profiles at high resolution around roots of living graminoids are required to prove that their root zones are indeed microenvironments with higher \(\text{H}_2\) availabilities. Thus far, in situ \(\text{H}_2\) concentrations could only be resolved at larger scales at fen Schlöppnerbrunnen (Knorr et al., 2009; Estop-Aragonés et al., 2013). (3) Product profiles in unsupplemented soil treatments corroborated the assumption that in the absence of root-derived organic carbon, acetogens are outcompeted for endogenous \(\text{H}_2\) by \(\text{H}_2\) consumers with lower thresholds. In the iron rich fen Schlöppnerbrunnen, not only hydrogenotrophic methanogens (e.g., \(\text{Methanocellales}\) and \(\text{Methanobacterium}\)) but also iron reducers (e.g., \(\text{Geobacter}\)) and, presumably, sulfate reducers compete with acetogens for available \(\text{H}_2\) (Reiche et al., 2008; Hausmann et al., 2016). However, acetogens are metabolically flexible and do not rely on \(\text{H}_2\) (Drake et al., 2008; Schuchmann and Müller, 2016). In situ, acetogens could thrive on root-derived organic carbon, organic fermentation products, and \(\text{CO}\) in addition to \(\text{H}_2\). In this respect, acetogens can co-metabolize several energy sources (e.g., \(\text{H}_2\) and formate) at the same time or grow mixotrophically (i.e., \(\text{H}_2\) is used as a lithotrophic energy source and organic compounds are used as heterotrophic carbon source). Furthermore, acetogens are well adapted to changing redox conditions that are characteristic for the root-zone of wetland plants (Conrad, 1996; Brune et al., 2000). Thus, when \(\text{O}_2\) and alternative electron acceptors are temporarily and locally depleted, acetogens might dominate \(\text{H}_2\) oxidation until hydrogenotrophic methanogens reestablish \(\text{H}_2\) concentrations below the threshold of acetogens.

**Conclusion**

The root-zones of fen graminoids are hotspots for \(\text{H}_2\)-producing fermenters in the fen Schlöppnerbrunnen, and it was hypothesized that acetogens may thrive on \(\text{H}_2\) diffusing into the soil around graminoid roots (Hunger et al., 2016; Meier et al., 2021). In the present study, potential acetogenic phyotypes successfully competed with methanogens in soil and root treatments when \(\text{H}_2\) was available in sufficiently high concentrations. However, acetogens and methanogens were outcompeted, possibly by iron reducers, when \(\text{H}_2\) concentrations were low (unsupplemented root-free soil treatments). To prove that acetogens can indeed thrive on \(\text{H}_2\) in the root-zones of graminoids in the fen Schlöppnerbrunnen and other peatlands, radial \(\text{H}_2\) profiles at high resolution around roots of living plants would be required. Nevertheless, especially those acetogens that are more tightly associated to the roots (e.g., acetogenic phyotypes that were detected in root-treatments; Table 4) could thrive directly on root-derived organic carbon or organic fermentation products (e.g., ethanol) in addition to \(\text{H}_2\).

By shifting the flow of carbon and reductant toward acetate during the anaerobic degradation of root organic carbon, hydrogenotrophic and organotrophic acetogens collectively can limit the \(\text{H}_2\) availability for hydrogenotrophic methanogens (Conrad, 1999; Schuchmann and Müller, 2016). When acetate is subsequently not consumed by aceticlastic methanogens, as observed in a peatland in Alaska (Duddleston et al., 2002), the overall production of \(\text{CH}_4\) is low. Thus, acetogens in the root zone of graminoids could be involved in controlling \(\text{CH}_4\) production in some peatlands, and further studies are needed to better resolve trophic links between acetogens and acetate consuming prokaryotes in these globally relevant ecosystems.

**Data availability statement**

The data presented in the study are deposited in the European Nucleotide Archive (ENA) repository, accession numbers: PRJEB37304 and PRJEB37863; the data have been released.

**Author contributions**

AM, SO, HD, and OS conceived to the study. AM performed the experiments. AM and OS analyzed the data. OS wrote the manuscript with input from all authors. All authors have read and agreed to the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.978296/full#supplementary-material

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