

Microbial Community Structure and Function in Peat Soil

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Summary

Many peatlands in Europe have been subjected to land reclamation and systematic drainage, which have substantially affected nutrient cycles in the soil. This work reviews published studies on microbial processes linked to carbon and nitrogen transformations in the soils of the Ljubljana marsh, a drained peatland positioned close to Ljubljana, the capital of Slovenia. This region is known for its dramatic diversity of animal and plant life, but below ground it hides diverse bacterial and archaeal communities that are highly responsive to environmental changes and make the Ljubljana marsh soils a good source of N₂O and CO₂, and a sink for CH₄. Methanogenesis is highly restricted in these soils due to competition for electron donors with iron reducers. In addition, methane is efficiently removed by methanotrophs, which are highly active, especially in the soil layers exposed to the changing water table. Denitrification is limited by electron acceptors and in deeper soil layers also by carbon, which becomes more recalcitrant with depth. Nitrification involves bacterial and archaeal ammonia oxidisers with ammonia oxidation rates being among the highest in the world. Interestingly, ammonia-oxidising Thaumarchaeota in acidic bog soils thrive only on ammonia released through mineralisation of organic matter and are incapable of oxidising added mineral ammonia. The soils of the Ljubljana marsh are rich in bacterial laccase-like genes, which may encode enzymes involved in lignin degradation and are therefore interesting for bioexploitations. Future challenges involve designing studies that will reveal specific physiological functions of phenol oxidases and other enzymes involved in peat transformations and address relations between microbial diversity, function and ecosystem responses to anthropogenic disturbances.

Key words: microbial community, microbial diversity, peatland, greenhouse gas, nitrification, denitrification, methanogenesis, methanotrophy, laccase, *amoA*

Introduction

This article is a short review of the published studies performed at the University of Ljubljana in the last ten years that aimed to decipher the structure, diversity and function of bacterial and archaeal microbial communities in the Ljubljana marsh soils. This mire which covers approx. 16 000 ha has been subjected to systematic drainage by open ditches and agricultural use over the last 200 years. Drainage has been applied as the management strategy to change the land use and has strong-

ly influenced the water table regime. In addition, urbanisation of the area has contributed to the change of the landscape and to localised pollution of the groundwater by municipal discharge and by agriculture. Due to the unique diversity of animal and plant life, especially birds that are represented by at least 100 different species in this area, the Ljubljana marsh was proclaimed a protected area by Natura 2000. This drained marsh is also of agricultural importance and highly important as the drinking water reservoir for the nearby capital, Ljubljana.

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While plant and animal diversity has been studied extensively for decades, microbiology of these carbon-rich peat soils has been unknown until recently. Our research over the last ten years has focused on soil microbial communities, their composition, diversity and function in carbon and nitrogen cycles. We have particularly been interested in the changes of bacterial and archaeal communities, and how specific functional guilds respond to specific environmental factors (*e.g.* soil type, pollution, temperature, water regime).

First, we shall describe our study sites in the Ljubljana marsh and then cover the findings revealing: (i) relationships between bacterial community structure and activity in respect to soil organic carbon (SOC) content and soil pH (1,2); (ii) microbial processes involved in soil organic matter (SOM) mineralisation, greenhouse gas (GHG) emissions, and carbon (C) and nitrogen (N) cycles in different water and temperature regimes (3–6); (iii) the composition, activity and function of ammonia-oxidising bacteria and archaea in relation to soil depth, soil type, pollution and SOM mineralisation (7–9). Finally, we will address the diversity of bacterial enzymes (laccase-like oxidoreductases) in two types of soil (10) that are believed to contribute to SOM mineralisation including peat (11). Our studies were the first to address the structure-function relationships of microbial communities in the Ljubljana marsh, but their relevance is broader and applicable to microbial ecology of peat soils in general.

The Ljubljana Marsh – A Peatland Ecosystem

Peatlands are ecosystems with layers of partially decomposed remains of dead plants that have accumulated in waterlogged areas for thousands of years. Due to the changing water table, which is often close to or even above the land surface, they are classified as wetlands, with anoxia commonly present in the deeper layers of the ecosystem (12). These lands represent 5–6 % of continental surface in Europe and are distributed in northern and temperate zones (13), while the Ljubljana marsh covers about 0.8 % of Slovenian territory. Peatlands are estimated to store up to a third of all the terrestrial carbon, and sequestration of carbon arises from an imbalance between plant input (primary production) and loss through microbial decomposition. Peat soils are rich in organic matter and are typically dark-brown or almost black. There are two types of peatlands: fens and bogs. The two are distinguished based on the water supply, which in fens originates from the mineral-rich groundwater and in bogs from the mineral-poor rainwater. Bog soils are covered with *Sphagnum* sp. and therefore have a low pH value, while fens are slightly alkaline with pH values between 7 and 8.

Soil organic matter (SOM) is essential to many ecosystem functions, and understanding its dynamics and factors driving its mineralisation is a key to understanding the soil productivity and its role as a sink and source of GHG (14). Specific conditions found in peatland ecosystems, such as low oxygen availability due to a high water table, cold temperatures which are prevalent in the northern peatlands, acidity and oligotrophy, all contribute to the composition and diversity of microbial com-

munities (12). However, relationships among environmental factors, microbial community composition and ecosystem function are still understudied. Furthermore, change of land use associated with the application of drainage systems affect SOM mineralisation, GHG fluxes and other processes in these ecosystems.

Studies of the Ljubljana marsh have been performed in the last ten years on sites that differ in soil type and groundwater level: the drained fen grassland soils (45°58'N, 14°28'E) and two bog forest soils, drained and undrained (45°59'N, 14°30'E). The fen site was grassland with the grass cut once or twice annually with dominating species *Arrhenatherum elatius*, *Dactylis glomerata*, *Festuca rubra*, *Equisetum palustre* and *Galium mollugo*, followed by *Ranunculus repens*, *Achillea millefolium*, *Leucanthemum ircutianum* and *Centaurea jacea* (15). The region of the grassland closer to the main drainage ditch had fluvial clay deposits with lower organic carbon content (LC) and lower groundwater level. On the other hand, the region distal from the main drainage ditch had no clay deposits, higher organic carbon content (HC) and higher groundwater level. These two fen sites were classified as rheic fibric histosol (HC) and humic gleysol (LC) (16). The bog site was a forested area representing a natural succession with *Betula* sp., *Frangula alnus*, *Salix* sp., *Quercus robur* and *Pinus sylvestris* as dominating tree species in the drained and undrained regions (17). The bottom layer vegetation in the undrained region (KG1) was dominated by *Sphagnum* sp. and other mosses, *Calluna vulgaris* and some herbaceous species, whereas the bottom layer vegetation in the drained region (KG2) consisted exclusively of *Pteridium aquilinum* (Hacin, unpublished data). The undrained bog soil (KG1) was classified as the rheic fibric histosol (dystric), while the drained bog soil (KG2) was classified as rheic hemic histosol (dystric) (3). All these sites differ significantly in many physicochemical parameters described in Table 1.

Activity and Diversity of Microbial Communities in Peat Soil

Microorganisms represent the most diverse group of organisms on Earth and they are the main drivers of the carbon and nitrogen cycles. A typical gram of soil has been estimated to contain 10^4 bacterial species and 10^9 microbial cells (18). Understanding the mechanisms regulating this vast diversity and the influence of these mechanisms on the ecosystem processes is central to ecology (19). The rapid advancement of molecular methods in the last two decades has enabled addressing of the relationships among microbial community structure, diversity and ecosystem function in the Ljubljana marsh soils (1,4,5,7,10).

Numerous studies have addressed the diversity and composition of microbial communities in soils including peat soils (reviewed in 12). However, despite major advances in molecular methods that have been applied to study microorganisms in the soil, our understanding of community structure/function relationship and relative contributions of various microbial groups to SOM transformations and N cycle remains limited. Kraigher *et al.* (1) and Ausec *et al.* (2) explored relationships in microbial activity and structure in two fen types (HC and LC)

Table 1. Soil characteristics from two fen grassland and two bog forest sites in the Ljubljana marsh, Slovenia (3)

	Study sites			
	Fen grassland		Bog forest	
	HC (drained)	LC (drained)	KG1 (undrained)	KG2 (drained)
$w(C_{org})/\%$	16.3±0.2	9.7±0.1	45.4±0.2	40.4±0.8
$w(N_{org})/\%$	1.4±0	0.88±0.01	2.75±0.01	2.09±0
C/N ratio	11.7±0.2	11.1±0.2	16.5±0.01	19.3±0.4
pH (in water)	7.55±0.02	7.63±0.01	4.58±0.2	4.3±0.1
WHC	1.6±0.2	1.4±0	8.1±0.3	4.8±0.4
Bulk density/(g/cm)	0.59±0.05	0.74±0.04	0.16±0.05	0.21±0.01
Groundwater level/cm	53±22	97±14	24±14	557±16
CO ₂ flux/(mg/(m ² -h))	200±29	206±28	139±26	204±34
N ₂ O flux/(mg/(m ² -h))	0.6±0.2	0.4±0.1	0.6±0.2	1.0±0.4
CH ₄ flux/(mg/(m ² -h))	0.04±0.03	0.05±0.02	0.03±0.06	-0.04±0.05
Annual N ₂ O emission/(g/(m ² -year))	5.83±0.03	4.21±0.02	5.77±0.03	9.52±0.1
Annual CH ₄ emission/(g/(m ² -year))	0.31±0.01	0.3±0.01	0.31±0.01	-0.28±0.01

Values are means with standard deviations ($N=3$)

HC=rheic fibric histosol, LC=humic gleysol, KG1=rheic fibric histosol (dystric), KG2=rheic hemic histosol (dystric)

WHC=water holding capacity, $m(H_2O)/m(soil)/(g/g)$

and one bog type (KG1). Two fen soils differed in SOC and mineral nitrogen content, bulk density and water holding capacity (WHC) but not in C/N ratio and pH, while the bog soil had low pH (4.3) and approx. 3 times higher SOC and WHC than fen soils (Table 1). The influence of SOC content on microbial communities had been addressed before by others. For example, Drenovsky *et al.* (20) found organic carbon availability and water content to be major determinants of the composition of soil microbial community. Also, soil priming by labile carbon induced changes in microbial activity and the composition of the community with the most prominent changes in fungi and actinomycetes but without detectable shifts in the Gram-negative populations (14).

Substrate-induced respiration and total dehydrogenase activity measurements in two fen soils of the Ljubljana marsh indicated that microbial activity increased with the increased SOC content. However, the difference in SOC content was only reflected at the level of activity but not at the level of microbial community structures (1). The relationship with SOC content was less clear in the bog soil, where low pH probably restrained microbial activity despite higher SOC content (2). However, the composition of bacterial community of the bog soil was clearly different from the fen community. This is not surprising as pH has a major effect on the microbial community composition (21).

Sequencing of 16S rRNA genes revealed that the dominating species in fen and bog soils belonged to the phyla *Proteobacteria* and *Acidobacteria* (1,2), which are commonly identified as dominant phyla in soil (22–24). Despite sharing dominant phyla, bog and fen soils also showed a clear distinction in bacterial communities. A significant difference was observed at the level of relative abundance of species affiliated with the phylum *Acidobacteria* (23 and 40 % of sequences were affiliated with *Acidobacteria* in the fen and bog gene library, re-

spectively). Each of the phylogenetic subgroups of this phylum was retrieved in only one of the studied habitats. Consistently, unique microbial communities are found in different soil types in general and it is believed that the soil type (25), plant cover and specific physicochemical characteristics (26) are the major determinants of the composition of a microbial community. While community composition clearly differed between fen and bog, the microbial diversity was rather similar in both soils despite their different chemical and physical parameters (2). This is in contrast with the general trend observed by Fierer *et al.* (21), who found that pH was the best predictor of the microbial diversity in soil, with the lowest values in acidic soils. Similarly, Lin *et al.* (27) found that bacterial richness was more than two times higher on average in the fen than in the bog, which was attributed to higher pH, labile SOC content and enhanced activities of laccase and peroxidase enzymes in the fen.

Bacterial communities in fen soils next to three plant species (*Cruciata laevipes*, *Mentha piperita* and *Equisetum arvense*) in the Ljubljana marsh and the bulk, plant-free soil in the vicinity were also studied (28). The terminal restriction fragment length polymorphism analysis indicated a different bacterial community structure in the rhizosphere and in bulk soil. However, no seasonal changes between late spring and autumn samples and no apparent impact of the three plant species were observed. The three plant species were also colonized by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE). Similarly, no specific effects of either plant species or seasonal changes on mycorrhizal community structure were observed using the temporal temperature gradient gel electrophoresis of both 18S-rDNA and the 5.8S-ITS2-28S fungal rDNA PCR products, again suggesting rather stable microbial communities in these drained fen soils.

Microorganisms as Drivers of Greenhouse Gas Emissions in Peat Soil

Wetlands including peatlands are important players in carbon and nitrogen cycling. It is estimated that one third of the total SOC is preserved in the northern peatlands, where it has accumulated since the ice age (29). Most of the wetlands including peatlands are net carbon sinks and in addition provide many ecosystem services (30). These lands are also important contributors to GHG emissions, which are produced during SOM mineralisation. Extensive drainage of wetland ecosystems stirred up a global concern of wetlands becoming GHG sources rather than sinks (31). In general, methane is a dominant GHG formed in natural peatlands with high groundwater level (32), while drained peatlands can be considerable contributors to the emissions of CO₂ (33,34) and N₂O (35).

The influence of soil type (fen, bog) and environmental factors (temperature, groundwater level) on GHG emissions (CO₂, CH₄ and N₂O) from the Ljubljana marsh soils was studied (3). GHG emissions were monitored weekly or biweekly *in loco* over a one-year period at two fen and two bog sites using the static chamber technique (35–37). The lowest CO₂ fluxes occurred during winter, while N₂O fluxes were the highest during summer and early spring (February, March) and CH₄ fluxes were highest during autumn (3). The observed pattern in CO₂ fluxes correlated with temperature, whereas CH₄ and N₂O fluxes were responsive to the groundwater level and soil carbon content. The study sites of Ljubljana marsh can therefore be considered as the net sources of GHG except for the drained bog site, which was determined as a net sink for CH₄ (3). CO₂ and CH₄ fluxes in the Ljubljana marsh soils are similar to those measured in the bog peatlands of Central and Northern Europe (33,34,38); however, cumulative N₂O emitted during our study was significantly higher (33,34). Exponential relationships between soil C/N ratio and annual N₂O emissions from similar soils had been modelled previously (39,40). The N₂O emissions from the Ljubljana marsh measured at drained fen sites with similar C/N ratio (HC and LC) could be described by the model of Ernfors *et al.* (40), while at undrained and drained bog sites (KG1 and KG2) the emissions were 9- and 25-fold, respectively, higher than predicted by the model. Inconsistency with the C/N ratio model was explained by the lower groundwater level, different carbon availability, higher mean annual temperature and higher precipitation (3). We believe that these environmental conditions contribute to dramatic annual N₂O emissions from the Ljubljana marsh.

Laboratory studies with soil microcosms exposed to different temperatures (4 and 28 °C) and water content (45 and 90 % WHC) for 120 days provided further insights into CO₂ and N₂O emissions (4) and microbial methane cycling (4,5) (Fig. 1). Microbial respiration in fen soils of the Ljubljana marsh increased with temperature but it was not affected by the soil water content. The N₂O production was also the highest under warm and wet regimes (4). In contrast, methane was only detected in these soils after a prolonged lag phase, even in water-logged soils or in soils incubated in the anaerobic atmosphere (4,5). In the Ljubljana marsh, methane molar fraction in the pore air below the groundwater fluctua-

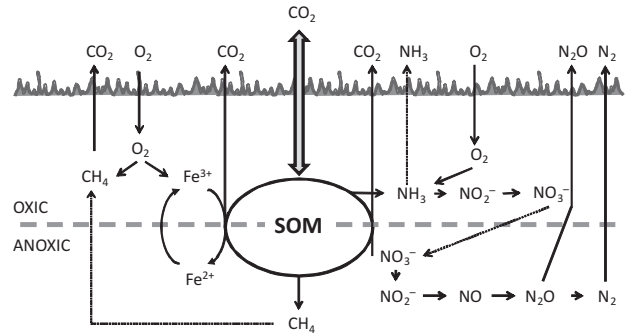


Fig. 1. A schematic overview of the main aerobic (oxic) and anaerobic (anoxic) microbial processes in the studied peat soil. This figure summarizes our insights into methanogenesis, methanotrophy, soil organic matter (SOM) mineralisation, nitrification and denitrification in the Ljubljana marsh

tion was low. It decreased with soil depth and remained lower than 0.005 %. Anaerobic incubations of soil slurries showed delayed methane production in all soil layers, with the shortest lag phase in the upper 10-cm layer. This means that SOM was more recalcitrant in deeper soil layers (41) and that methanogenesis was affected by SOM quality, as suggested previously for paddy soils (42). However, the availability of alternative electron acceptors may also have an important impact on methane production (43) as methanogenesis is generally the last process in the anaerobic degradation of organic matter. Fermentation of organic polymers controls the speed of subsequent catabolic processes, and the activity of syntrophic communities involving methanogenes is affected by partial pressure of hydrogen and by the availability of other methanogenic substrates. Fermentation products are also targeted by competing microbial groups like iron reducers (44). For example, in the Ljubljana marsh soils, Fe(III) reducers, like *Geobacter* species, compete with the acetoclastic methanogens, which dominate over hydrogenotrophs (5). These results also explain, at least in part, a prolonged lag phase for methane production in anaerobic soil microcosms (4,5), and unusually low methane emissions observed by Danevčič *et al.* (3). In addition, poor availability of SOM, especially in deeper layers of the soil further restricts methanogenesis. During the cold season and in deeper soil layers methanogens are additionally limited by temperature, as the optimum temperature for methanogenesis is rather high (37 °C) in fen soils. This optimum is higher than the one in northern peatlands, but similar to optima for methanogenesis of the rice fields in northern Italy (45). Finally, in these soils methane-oxidising bacteria are highly active and abundant throughout the soil profile, which may additionally limit CH₄ emissions. Methanotrophs that inhabit the Ljubljana marsh fen soils are presumably adapted to low concentrations of methane (41) or are mixotrophs, as suggested previously for *Methylococcus capsulatus* (46). However, further studies are needed to test these predictions.

Microorganisms as Drivers of Nitrogen Cycle in Peat Soil

The Ljubljana marsh represents an important supply of drinking water for the Ljubljana region (47), yet these

soils are under intense agricultural use, with annual fertiliser application and household and farm municipal waters discharged into septic tanks, which also represent pollution sources. Therefore, knowledge on key microbial groups and their role in the N transformation processes, such as nitrification (6–9) and denitrification (4,6), is important for understanding their impact on environmental pollution (Fig. 1). Denitrification is an anaerobic respiratory process that couples SOM oxidation to reduction of nitrate to nitrogen gasses (N_2O and N_2), and many bacterial species are capable of this process. In organic soils of the Ljubljana marsh, nitrate concentrations were low but a potential for denitrification enzyme activity was high, especially in the upper 30 cm, where the rates were estimated to be 1.0–2.3 kg of N per hectare and day (6). The high potential for nitrate removal is important for preventing the nitrate from leaching into groundwater although denitrification potential decreases with soil depth (48). The HC fen soil was also tested for its long-term potential for nitrate and ammonium removal during oxic and anoxic cycles (6). We expected that due to high carbon content the soil would be able to sustain N removal even during repeated amendments. The results, however, showed that the efficiency of anoxic nitrate removal decreased with the number of cycles and ceased completely after the fifth anoxic cycle. Denitrification potential recovered only if external carbon sources were added to the soil microcosms, implying that denitrification could be limited by carbon even in this carbon-rich peat soil (6).

Bacterial autotrophic nitrification is a two-step process, carried out by chemolithotrophic ammonia- and nitrite-oxidising bacteria (AOB and NOB). The rate of nitrification in soil depends on ammonia and oxygen supplies (49). Until recently, ammonia oxidation, the first step of nitrification, was thought to be performed almost exclusively by bacteria (50,51). Recent metagenomic studies (52–54) and analysis of community structure, transcriptional activities and abundances of *amoA* genes (gene encoding ammonia monooxygenase enzyme subunit) in soil (55–57) have revealed the existence and possible involvement of archaea in ammonia oxidation (58).

The ammonia oxidisers in the Ljubljana fen and bog were strikingly different (7–9). AOB abundance, determined by qPCR-based enumeration of specific bacterial *amoA* genes, was below the detection limit in fen and bog but it increased to detectable numbers in nitrogen-enriched soils. In fen soil, for example, nitrification and growth of AOB was stimulated by the pollution of soil through septic tank leakage (7). On the other hand, neither addition of organic nor inorganic N sources could stimulate the growth of AOB in the bog, where AOT (ammonia-oxidising Thaumarchaea) were exclusive *amoA*-containing organisms and presumably the only organisms to carry out the first step of nitrification in this acidic environment (pH=4.3). Interestingly, their activity was unaffected by the addition of inorganic sources of N (8,9) and only constant and slow supply of ammonium through SOM mineralisation fed these ammonia-oxidising archaea in bog soils (8). As nitrification is significantly stimulated by the addition of organic N (9), this ecosystem is probably oligotrophic. In fact, others also showed that AOT may outcompete bacteria under the

conditions of low-energy supply, such as are found in low-ammonium soils, suboxic wastewater plants and oligotrophic waters (55,59,60). Therefore, for natural wetlands, including the Ljubljana marsh, the ratio of AOT/AOB could serve as an indicator of oligotrophic *vs.* eutrophic conditions, as suggested previously by Sims *et al.* (61). Furthermore, findings by Levičnik-Höfferle *et al.* (9) also suggest a niche specialisation for ammonia oxidisers, with AOT utilising organic and AOB inorganic sources of nitrogen. Mixotrophic metabolism of Thaumarchaea present in the bog is also possible, but the question awaits further investigation.

The Ljubljana marsh is subjected to annual fluctuations in groundwater level, due to heavy rain and occasional flooding (4,16). The vertical soil profile is generally characterised by changes in the soil redox potential and oxygen availability (62), resulting in different mineralisation rates and organic matter quality (63). Interestingly, a strong stratification of AOT community at different depth of HC fen soil (7) suggested that some AOT ecotypes may be more adapted to low-oxygen or almost anoxic conditions and others to more oxic conditions, as speculated previously (64). On the other hand, in many soils the ammonia release following mineralisation of organic matter controls the nitrification rate (65). In HC fen soil, SOM mineralisation measured by CO_2 production rate is oxygen dependent, but also occurs under anoxic conditions (4,6). Furthermore, occasional fluctuations of groundwater levels may enable some oxygen diffusion to the deeper, mostly submerged layers, stimulating mineralisation and providing slow, but sufficient supply of substrate ammonia and oxygen for low-energy-requiring AOT ecotypes (7).

Diversity of Genes Encoding for Laccase-Like Enzymes in Ljubljana Marsh

Laccases are oxidoreductases that couple the oxidation of different phenolic substances with the reduction of oxygen to water. They are versatile and environmentally friendly enzymes that have been most extensively studied in white-rot fungi, where they contribute to SOM mineralisation through oxidation of lignin components. In addition to their important function in the ecosystem, these enzymes are industrially important and utilised for example in the paper and textile industries (66). There is an ongoing effort to find laccases that would be more robust than the currently used fungal enzymes and that could even degrade lignin (67). Bacterial laccases potentially have several such properties as some of them are stable at extreme temperatures and pH values (68). However, bacterial laccase-like enzymes have not been studied enough, and their diversity and role in the ecosystem are practically unknown.

Until recently only one study has addressed the diversity of bacterial genes encoding for laccase-like enzymes in forest soils, specifically in the humic horizons (69). We utilised the approach applied by Kellner *et al.* (69) to examine the diversity of laccase-like genes in fen and bog of the Ljubljana marsh (10). The phylogenetic analysis of short gene fragments (<150 bp) indicated 11 major clusters with one third of the sequences resem-

bling the laccase-like genes of *Acidobacteria*. To extend the study, we designed a new PCR primer that allowed the amplification of large portions of the bacterial laccase genes. This enabled us to address the diversity of bacterial genes encoding for laccase-like enzymes in greater depth, retrieving genes encoding for the conventional three-domain and the more recently recognised two-domain laccases (70). Our work brought evidence that laccase-like sequences were highly diverse in the Ljubljana marsh with little or no similarity to the sequences in the public databases (10). The soil was abundant with acidobacterial genes encoding both three- and two-domain laccases (10). The diversity of laccase-encoding genes showed the same trend as the diversity of the 16S rRNA genes examined previously (1,2), again establishing *Acidobacteria* as the dominant and ecologically important group of bacteria in the peat soil of the Ljubljana marsh.

Conclusions and Future Challenges

The Ljubljana marsh shows high biological diversity above ground but also a comparable, if not even greater biodiversity below ground, which is represented by the highly diverse bacterial and archaeal communities of acidic (bog) and neutral (fen) peat soils. The diversity is reflected at the level of 16S rRNA genes and specific functional genes like *amoA* or genes encoding bacterial laccases. These microorganisms perform important functions in the ecosystem through SOM mineralisation, which is linked to C and N cycles. They are adapted to highly changing environments brought about by a changing groundwater level and seasonal changes. In the fen soils respiration was mainly affected by temperature and less so by waterlogged conditions. The CO₂ and CH₄ emissions were comparable to the emissions measured in other drained peatlands. The Ljubljana marsh showed very low methane emissions, which can be explained by a frequently changing water table, a high amount of iron in the soil that supports the growth of competitors and by highly active methanotrophy. Although the Ljubljana marsh is covered with soils with high content of organic carbon, processes such as denitrification or methanogenesis may be limited by carbon, which is in recalcitrant form especially in the deeper layers of the peatland. In contrast to the expected CO₂ and CH₄ fluxes, the N₂O emissions were approximately ten times higher in this area compared to other peatlands. The reason for this is not understood. These soils have very active nitrification and especially ammonia-oxidising activity, which is largely dependent on AOT and on the ammonia released through SOM mineralisation. The conversion of ammonia to nitrate by nitrification then drives denitrification in waterlogged soils and other anaerobic microniches. A tight coupling between nitrification and denitrification might at least partially explain the dramatically high N₂O emissions in the low nitrate peat soils.

Investigations of soil microbial ecology in the Ljubljana marsh revealed exciting diversity of microbial life, uncovered novel niche preferences of archaeal ammonia oxidisers and have for the first time shown that soil bacteria encode two- and three-domain laccase-like genes. The work has broad implications for other drained peat-

lands and represents the basis for the application of the findings for better management of this unique and dynamic peatland.

References

1. B. Kraigher, B. Stres, J. Hacin, L. Ausec, I. Mahne, J.D. van Elsas, I. Mandić-Mulec, Microbial activity and community structure in two drained fen soils in the Ljubljana Marsh, *Soil Biol. Biochem.* 38 (2006) 2762–2771.
2. L. Ausec, B. Kraigher, I. Mandić-Mulec, Differences in the activity and bacterial community structure of drained grassland and forest peat soils, *Soil Biol. Biochem.* 41 (2009) 1874–1881.
3. T. Danevčič, I. Mandić-Mulec, B. Stres, D. Stopar, J. Hacin, Emissions of CO₂, CH₄ and N₂O from Southern European peatlands, *Soil Biol. Biochem.* 42 (2010) 1437–1446.
4. B. Stres, T. Danevčič, L. Pal, M. Mrkonjić Fuka, L. Resman, S. Leskovec *et al.*, Influence of temperature and soil water content on bacterial, archaeal and denitrifying microbial communities in drained fen grassland soil microcosms, *FEMS Microbiol. Ecol.* 66 (2008) 110–122.
5. V. Jerman, M. Metje, I. Mandić-Mulec, P. Frenzel, Wetland restoration and methanogenesis: The activity of microbial populations and competition for substrates at different temperatures, *Biogeosciences*, 6 (2009) 1127–1138.
6. L. Pal, B. Stres, T. Danevčič, S. Leskovec, I. Mandić-Mulec, Transformations of mineral nitrogen applied to peat soil during sequential oxic/anoxic cycling, *Soil Biol. Biochem.* 42 (2010) 1338–1346.
7. Š. Höfferle, G.W. Nicol, L. Pal, J. Hacin, J.I. Prosser, I. Mandić-Mulec, Ammonium supply rate influences archaeal and bacterial ammonia oxidizers in a wetland soil vertical profile, *FEMS Microbiol. Ecol.* 74 (2010) 302–315.
8. N. Stopnišek, C. Gubry-Rangin, Š. Höfferle, G.W. Nicol, I. Mandić-Mulec, J.I. Prosser, Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment, *Appl. Environ. Microbiol.* 76 (2010) 7626–7634.
9. Š. Levičnik-Höfferle, G.W. Nicol, L. Ausec, I. Mandić-Mulec, J.I. Prosser, Stimulation of thaumarchaeal ammonia oxidation by ammonia derived from organic nitrogen but not added inorganic nitrogen, *FEMS Microbiol. Ecol.* 80 (2012) 114–123.
10. L. Ausec, J.D. van Elsas, I. Mandić-Mulec, Two- and three-domain bacterial laccase-like genes are present in drained peat soils, *Soil Biol. Biochem.* 43 (2011) 975–983.
11. C. Freeman, C.D. Evans, D.T. Monteith, B. Reynolds, N. Fenner, Export of organic carbon from peat soils, *Nature*, 412 (2001) 785.
12. R. Andersen, S.J. Chapman, R.R.E. Artz, Microbial communities in natural and disturbed peatlands: A review, *Soil Biol. Biochem.* 57 (2013) 979–994.
13. M. Drösler, A. Freibauer, T.R. Christensen, T. Friborg: Observations and Status of Peatland Greenhouse Gas Emissions in Europe. In: *The Continental-Scale Greenhouse Gas Balance of Europe, Ecological Studies, Vol. 203*, A.J. Dolman, A. Freibauer, R. Valentini (Eds.), Springer, New York, NY, USA (2008) pp. 243–261.
14. J. Garcia-Pausas, E. Paerson, Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon, *Soil Biol. Biochem.* 43 (2011) 1874–1881.
15. J. Čop, T. Sinkovič, M. Vidrih, J. Hacin, Influence of cutting and fertilising management on the botanical composition of Ljubljana marsh grasslands, *Acta Agr. Slov.* 83 (2004) 157–169 (in Slovene).

16. J. Hacin, J. Čop, I. Mahne, Nitrogen mineralization in marsh meadows in relation to soil organic matter content and watertable level, *J. Plant Nutr. Soil Sci.* 164 (2001) 503–509.
17. A. Martinčič, Fragments of bog soil from Ljubljana marsh, *Scopolia*, 14 (1987) 1–53 (in Slovene).
18. R. Roselló-Mora, R. Amann, The species concept for prokaryotes, *FEMS Microbiol. Rev.* 25 (2001) 39–67.
19. G.M. Mace, K. Norris, A.H. Fitter, Biodiversity and ecosystem services: A multilayered relationship, *Trends Ecol. Evol.* 27 (2012) 19–26.
20. R.E. Drenovsky, D. Vo, K.J. Graham, K.M. Scow, Soil water content and organic carbon availability are major determinants of soil microbial community composition, *Microbial Ecol.* 48 (2004) 424–430.
21. N. Fierer, J.A. Jackson, R. Vilgalys, R.B. Jackson, Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays, *Appl. Environ. Microb.* 71 (2005) 4117–4120.
22. J.Z. Zhou, B.C. Xia, H.S. Huang, D.S. Treves, L.J. Hauser, R.J. Mural *et al.*, Bacterial phylogenetic diversity and a novel candidate division of two humid region, sandy surface soils, *Soil Biol. Biochem.* 35 (2003) 915–924.
23. D.A. Lipson, S.K. Schmidt, Seasonal changes in an Alpine soil bacterial community in the Colorado Rocky Mountains, *Appl. Environ. Microbiol.* 70 (2004) 2867–2879.
24. N. Fierer, R.B. Jackson, The diversity and biogeography of soil bacterial communities, *Proc. Natl. Acad. Sci. USA*, 103 (2006) 626–631.
25. H. Juottonen, P.E. Galand, E.S. Tuittila, J. Laine, H. Fritze, K. Yrjälä, Methanogen communities and bacteria along an ecohydrological gradient in a northern raised bog complex, *Environ. Microbiol.* 7 (2005) 1574–1557.
26. K. Opelt, C. Berg, S. Schonman, L. Eberl, G. Berg, High specificity but contrasting biodiversity of *Sphagnum*-associated bacterial and plant communities in bog ecosystems independent of the geographical region, *ISME J.* 1 (2007) 502–516.
27. X. Lin, S. Green, M.M. Tfaily, O. Prakash, J.E. Konstantinidis, J.E. Corbett *et al.*, Microbial community structure and activity linked to contrasting biogeochemical gradients in bog and fen environments of the Glacial Lake Agassiz Peatland, *Appl. Environ. Microbiol.* 78 (2012) 7023–7031.
28. M. Likar, M. Regvar, I. Mandic-Mulec, B. Stres, H. Bothe, Diversity and seasonal variations of mycorrhiza and rhizosphere bacteria in the three common plant species at the Slovenian Ljubljana Marsh, *Biol. Fertil. Soils*, 45 (2009) 573–583.
29. E. Gorham, Northern peatlands: Role in the carbon cycle and probable responses to climatic warming, *Ecol. Appl.* 1 (1991) 182–195.
30. W.J. Mitsch, B. Bernal, A.M. Nahlik, Ü. Mander, L. Zhang, C.J. Anderson *et al.*, Wetlands, carbon, and climate change, *Landscape Ecol.* 28 (2013) 583–597.
31. K.A. Smith, K.E. Dobbie, B.C. Ball, L.R. Bakken, B.K. Sitaula, S. Hansen *et al.*, Oxidation of atmospheric methane in northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink, *Glob. Change Biol.* 6 (2000) 791–803.
32. L.C. Smith, G.M. MacDonald, A.A. Velichko, D.W. Beilman, O.K. Borisova, K.E. Frey *et al.*, Siberian peatlands a net carbon sink and global methane source since the early Holocene, *Science*, 303 (2004) 353–356.
33. K. Von Arnold, M. Nilsson, B. Hånell, P. Weslien, L. Klemmedtsson, Fluxes of CO₂, CH₄ and N₂O from drained organic soils in deciduous forests, *Soil Biol. Biochem.* 37 (2005) 1059–1071.
34. K. Von Arnold, P. Weslien, M. Nilsson, B.H. Svensson, L. Klemmedtsson, Fluxes of CO₂, CH₄ and N₂O from drained coniferous forests on organic soils, *Forest Ecol. Manag.* 210 (2005) 239–254.
35. P.J. Martikainen, H. Nykänen, P. Crill, J. Silvola, Effect of lowered water table on nitrous oxide fluxes from northern peatlands, *Nature*, 366 (1993) 51–53.
36. H. Nykänen, J. Alm, K. Lång, J. Silvola, P.J. Martikainen, Emissions of CH₄, N₂O and CO₂ from a virgin fen and a fen drained for grassland in Finland, *J. Biogeogr.* 22 (1995) 351–357.
37. J. Alm, S. Saarnio, H. Nykänen, J. Silvola, P.J. Martikainen, Winter CO₂, CH₄ and N₂O fluxes on some natural and drained boreal peatlands, *Biogeochemistry*, 44 (1999) 163–186.
38. H.F. Jungkunst, S. Fiedler, Latitudinal differentiated water table control of carbon dioxide, methane and nitrous oxide fluxes from hydromorphic soils: Feedbacks to climate change, *Glob. Change Biol.* 13 (2007) 2668–2683.
39. L. Klemmedtsson, K. von Arnold, P. Weslien, P. Gundersen, Soil CN ratio as a scalar parameter to predict nitrous oxide emissions, *Glob. Change Biol.* 11 (2005) 1142–1147.
40. M. Ernfors, K. von Arnold, J. Stendahl, M. Olsson, L. Klemmedtsson, Nitrous oxide emissions from drained organic forest soils — An up-scaling based on C:N ratios, *Biogeochemistry*, 84 (2007) 219–231.
41. V. Jerman, Activity and structure of methanogenic archaea and methane oxidizing bacteria in the Ljubljana marsh soil, *PhD Thesis*, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia (2009) (in Slovene).
42. A. Hanke, C. Cerli, J. Muhr, W. Borken, K. Kalbitz, Redox control on carbon mineralization and dissolved organic matter along a chronosequence of paddy soils, *Eur. J. Soil Sci.* 64 (2013) 476–487.
43. P. Bergamaschi, P. Bousquet: Estimating Sources and Sinks of Methane: An Atmospheric View. In: *The Continental-Scale Greenhouse Gas Balance of Europe, Ecological Studies*, Vol. 203, A.J. Dolman, A. Fraibauer, R. Valentini (Eds.), Springer, New York, NY, USA (2008) pp. 113–133.
44. E.E. Roden, R.G. Wetzel, Competition between Fe(III)-reducing and methanogenic bacteria for acetate in iron-rich freshwater sediments, *Microb. Ecol.* 45 (2003) 252–258.
45. A. Fey, R. Conrad, Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil, *Appl. Environ. Microb.* 66 (2000) 4790–4797.
46. N. Ward, Ø. Larsen, J. Sakwa, L. Bruseth, H. Khouri, A.S. Durkin *et al.*, Genomic insights into methanotrophy: The complete genome sequence of *Methylococcus capsulatus* (Bath), *PLOS Biol.* 2 (2004) e303.
47. V. Ježovnik, Research of the subsidence of the Ljubljana marsh due to the drawing of water in the waterworks Brest, *Geodetski vestnik*, 53 (2009) 23–35 (in Slovene).
48. L. Pal, The potential of Ljubljana marsh soils for mineral nitrogen removal, *PhD Thesis*, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia (2011) (in Slovene).
49. G.P. Robertson, P.M. Groffman: Nitrogen Transformations. In: *Soil Microbiology, Ecology and Biochemistry*, E.A. Paul (Ed.), Academic Press, Boston, MA, USA (2007) pp. 341–364.
50. L.W. Belser, Population ecology of nitrifying bacteria, *Annu. Rev. Microbiol.* 33 (1979) 309–333.
51. J.I. Prosser, Autotrophic nitrification in bacteria, *Adv. Microb. Physiol.* 30 (1989) 125–181.
52. J.C. Venter, K. Remington, J.F. Heidelberg, A.L. Halpern, D. Rusch, J.A. Eisen *et al.*, Environmental genome shotgun sequencing of the Sargasso Sea, *Science*, 304 (2004) 66–74.
53. C. Schleper, G. Jurgens, M. Jonuscheit, Genomic studies of uncultivated archaea, *Nat. Rev. Microbiol.* 3 (2005) 479–488.

54. A.H. Treusch, S. Leininger, A. Kletzin, S.C. Schuster, H.P. Klenk, C. Schleper, Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic Crenarchaeota in nitrogen cycling, *Environ. Microbiol.* 7 (2005) 1985–1995.
55. S. Leininger, T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol *et al.*, Archaea predominate among ammonia-oxidizing prokaryotes in soils, *Nature*, 442 (2006) 806–809.
56. G.W. Nicol, S. Leininger, C. Schleper, J.I. Prosser, The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria, *Environ. Microbiol.* 10 (2008) 2966–2978.
57. M. Tourna, T.E. Freitag, G.W. Nicol, J.I. Prosser, Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms, *Environ. Microbiol.* 10 (2008) 1357–1364.
58. C.A. Francis, J.M. Beman, M.M.M. Kuypers, New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation, *ISME J.* 1 (2007) 19–27.
59. H.D. Park, G.F. Wells, H. Bae, C.S. Criddle, C.A. Francis, Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors, *Appl. Environ. Microbiol.* 72 (2006) 5643–5647.
60. C. Wuchter, B. Abbas, M.J.L. Coolen, L. Herfort, J. van Bleijswijk, P. Timmers *et al.*, Archaeal nitrification in the ocean, *Proc. Natl. Acad. Sci. USA*, 103 (2006) 12317–12322.
61. A. Sims, Y. Zhang, S. Gajaraj, P.B. Brown, Z. Hu, Toward the development of microbial indicators for wetland assessment, *Water Res.* 47 (2013) 1711–1725.
62. Z. Bohrerova, R. Stralkova, J. Podesvova, G. Bohrer, E. Pokorny, The relationship between redox potential and nitrification under different sequences of crop rotations, *Soil Till. Res.* 77 (2004) 25–33.
63. A. Konopka, R. Turco, Biodegradation of organic-compounds in vadose zone and aquifer sediments, *Appl. Environ. Microbiol.* 57 (1991) 2260–2268.
64. T.H. Erguder, N. Boon, L. Wittebolle, M. Marzorati, W. Verstraete, Environmental factors shaping the ecological niches of ammonia-oxidizing archaea, *FEMS Microbiol. Rev.* 33 (2009) 855–869.
65. M.S. Booth, J.M. Stark, E. Rastetter, Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data, *Ecol. Monogr.* 75 (2005) 139–157.
66. R. Couto, T.J.L. Herrera, Industrial and biotechnological applications of laccases: A review, *Biotechnol. Adv.* 24 (2006) 500–513.
67. T.D. Bugg, M. Ahmad, E.M. Hardiman, R. Singh, The emerging role for bacteria in lignin degradation and bio-product formation, *Curr. Opin. Biotechnol.* 22 (2011) 394–400.
68. R. Reiss, J. Ihssen, L. Thony-Meyer, *Bacillus pumilus* laccase: A heat stable enzyme with a wide substrate spectrum, *BMC Biotechnol.* 11 (2011) 9.
69. H. Kellner, P. Luis, B. Zimdars, B. Kiesel, F. Buscot, Diversity of bacterial laccase-like multicopper oxidase genes in forest and grassland Cambisol soil samples, *Soil Biol. Biochem.* 40 (2008) 638–648.
70. K. Nakamura, T. Kawabata, K. Yura, N. Go, Novel types of two-domain multi-copper oxidases: Possible missing links in the evolution, *FEBS Lett.* 553 (2003) 239–244.