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Interactions between Microorganisms and Oxic-Anoxic Transitions

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Summary

Oxygen depletion in waters may lead to hypoxia and anoxia, which are detrimental for most aerobic organisms. Although hypoxia and anoxia have occurred throughout geological time, the frequency, intensity and duration of hypoxia and anoxia in lakes, coastal waters and open oceans have increased during the past decades, most likely due to eutrophication and global warming. Oxygen consumption by microorganisms plays an important role in the development of hypoxia and anoxia and, vice versa, microbial activity is also strongly affected by changes in oxygen availability. In particular, many of the biogeochemical transformations mediated by microorganisms involve oxidation-reduction reactions. Hence, as hypoxia and anoxia are increasingly threatening aquatic ecosystems, it is imperative to understand the interactions between microorganisms and oxic-anoxic transitions. Therefore, this thesis investigates the diversity and dynamics of microbial communities during oxic-anoxic transitions in a seasonally stratified lake (Lake Vechten) in the Netherlands. The following research questions have been addressed:

(1) How do oxic-anoxic transitions affect bacterial community dynamics?

(2) How are microbial and chemical feedbacks involved in oxic-anoxic transitions?

(3) How do oxic-anoxic transitions affect the microbial sulfur and nitrogen cycle?

The influences of oxic-anoxic transitions on bacterial community dynamics in Lake Vechten were investigated in Chapter 2. *Cyanobacteria* and *Planktomycetes* were abundant throughout the water column in early spring. During summer stratification, heterotrophic *Alphaproteobacteria*, *Bacteroidetes* and *Actinobacteria* became abundant in the aerobic epilimnion, *Gammaproteobacteria* (mainly *Chromatiaceae*) dominated in the metalimnion, and *Chlorobi*, *Betaproteobacteria*, *Deltaproteobacteria* and *Firmicutes* were abundant in the

anoxic sulfidic hypolimnion. After fall turnover, the entire water column became hypoxic, *Polynucleobacter* (*Betaproteobacteria*) and *Methylobacter* (*Gammaproteobacteria*) spread out from the former meta- and hypolimnion to the surface layer, and *Epsilonproteobacteria* dominated in the bottom water layer. When the lake became fully mixed and oxic during the winter and early spring, *Cyanobacteria* and *Planktomycetes* dominated the bacterial community again. Overall, the bacterial community composition at different depths in the water column diverged during summer stratification and converged when the lake was mixed, indicating large spatio-temporal changes during oxic-anoxic transitions.

The interactions between microbial community composition, biogeochemical oxidation-reduction reactions and oxic-anoxic transitions were studied by a mathematical model in Chapter 3. The model predicts that gradual changes in oxygen influx can induce major regime shifts, in which the ecosystem shifts abruptly between an oxic state dominated by *Cyanobacteria* and an anoxic state with phototrophic sulfur bacteria and sulfate-reducing bacteria (SRB). Observations from Lake Vechten supported the model predictions, and showed hysteresis in the transition between oxic and anoxic states with similar changes in microbial community composition as predicted by the model. The hysteresis loops and tipping points associated with these regime shifts are likely a common feature of oxic-anoxic transitions in aquatic environments, causing rapid drops in oxygen levels that are not easily reversed. These results reveal and emphasize the vital roles of microorganisms in mediating oxic-anoxic transitions.

The dynamics of SRB and sulfur-oxidizing bacteria (SOB) during oxic-anoxic transitions were studied in detail in Chapter 4. SRB, green sulfur bacteria (GSB), purple sulfur bacteria (PSB), and colorless sulfur bacteria (CSB) inhabited the sediment during the winter and early spring when the water column was mixed. Once the water column stratified in late spring and summer, various SRB species expanded into the anoxic hypolimnion, and

PSB and GSB bloomed in the metalimnion and hypolimnion during summer. When hypoxia spread throughout the water column during fall turnover, SRB and GSB vanished from the water column, whereas CSB (mainly *Arcobacter*) and PSB (*Lamprocystis*) became dominant. They oxidized the sulfide that had accumulated in the hypolimnion during summer stratification. These results support the view that, once ecosystems have become anoxic and sulfidic, a large oxygen influx is needed to overcome this state and bring the ecosystem back into the oxic state.

In Chapter 5, the seasonal succession of microorganisms involved in the nitrogen cycle during oxic-anoxic transitions was investigated. Ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and anaerobic ammonium-oxidizing (anammox) bacteria were abundantly present in the sediment during the winter period. Nitrogen-fixing bacteria and denitrifying bacteria increased in the water column in spring, when nitrate was gradually depleted and the hypolimnion became anoxic. Denitrifying bacteria containing *nirS* genes were exclusively present in the anoxic hypolimnion. During summer stratification, abundances of AOA, AOB and anammox bacteria decreased in the sediment. After the lake was mixed during fall turnover, AOA, AOB and anammox bacteria increased to high abundances again. In general, nitrogen microorganisms in the water column and sediment displayed a pronounced seasonal succession, which was closely linked to the oxic-anoxic transitions.

Remaining questions on the diversity and functioning of microbial communities that are likely of interest for future research were discussed in Chapter 6. For instance, further efforts need to be made to assess links between the microbial sulfur and nitrogen cycle. Activity and diversity of microorganisms involved in the sulfur and nitrogen cycle and other important biogeochemical cycles (e.g., the carbon cycle) should be explored in further detail. Furthermore, accurate prediction of tipping points during oxic-anoxic transitions in lakes and coastal water will require refined quantification of microbially-mediated oxidation-reduction reactions in biogeochemical cycles. Preliminary results from metagenomics and metatranscriptomics analysis of samples collected from different water layers and the sediment of Lake Vechten indicate that these approaches can expand our understanding of microbial diversity and activity. For instance, the metatranscriptome revealed high activities of *Euryarchaeota* and *Chloroflexi* in the anoxic hypolimnion, whereas our earlier 16S rRNA gene analysis had not detected *Euryarchaeota* and only indicated a very low relative abundance of *Chloroflexi*.

Overall, this thesis advances our knowledge of dynamic changes in microbial community composition (especially of microorganisms involved in the sulfur and nitrogen cycle) in seasonally stratified lakes. In particular, our results show that the composition of microbial communities does not only track seasonal changes in environmental conditions, but also affects and modifies the environment through the involvement of microorganisms in biogeochemical oxidation-reduction processes. This interplay between biogeochemical processes and microbial community composition causes pronounced 'oxic-anoxic regime shifts', with drastic changes in the structure and functioning of lake microbial communities during oxic-anoxic transitions. Hence, the information in this thesis may contribute to an improved understanding and prediction of the major impact of microorganisms on the sulfur and nitrogen cycle and the development of hypoxia and anoxia in aquatic ecosystems.