Anoxic sediments and their potential to favour bacterial wood decay

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultäten

der Georg-August-Universität zu Göttingen

vorgelegt von

Ev Iris Kretschmar

aus Berlin-Mitte

Göttingen 12.06.2006

D 7

Referent: Herr Prof. Dr. G. Gerold

Korreferent: Herr Prof. Dr. F. Beese

Tag der mündlichen Prüfung: 10.07.2006

Meiner Mutter gewidmet

Summary

Wooden pile foundations are widely used along coastal areas and river sites in Europe to support historic buildings. They are in service to stabilise constructions in areas with soils of low bearing capacity. As well as many outstanding monuments, such as the parliament building in Stockholm and the 'Reichstag' in Berlin, nearly all of the buildings of Amsterdam, Rotterdam, Haarlem (The Netherlands) and Venice (Italy) rest on foundations comprising wooden piles. When the ground water level is low these wooden structures are exposed to air and they can be attacked by fungi causing degradation, unless they are protected from oxygen contact. Fungal degradation is prevented as long as the wooden constructions remain water logged, when oxygen availability is strongly reduced or anoxic conditions prevail. Under waterlogged conditions, however, bacteria can colonize the wood, leading to a certain degree of decay. Until the 1970's, bacterial wood decay was considered to be an extremely slow process, threatening only archaeological wooden specimens, such as shipwrecks, building remains or tools. Although bacterial wood degradation is slow compared to that caused by fungi, recent observations from The Netherlands and Sweden showed that bacterial activity under anoxic conditions can cause considerable strength loss of wooden foundations within a time span of one hundred years, endangering modern constructions. Existing knowledge on the species involved and the conditions required for growth of the bacteria is insufficient.

The main aim of the presented investigation was to characterise under which environmental conditions bacterial wood decay occured in foundation piles. In particular, we investigated: 1. If the presence of oxygen is a prerequisite for the bacterial wood decay process. 2. If elevated nitrogen concentrations due to eutrophication in the wood surroundings are favouring bacterial wood decay. 3. If CO_2 can serve as alternative bacterial wood decay detection method in laboratory experiments. In order to answer these questions three different investigations were conducted: Part I+II monitoring and sampling at foundation sites and Part III a laboratory experiment which divided into tree different parts itself.

Sediment samples were analysed on total contents of carbon, nitrogen, sulphur, phosphate and major cations. In sediment water samples pH, conductivity, and total and dissolved carbon and nitrogen, NH_4^+, NO_3^-, SO_4^{2-}, PO_4^{3-}, Cl^- and mayor cations were measured. Redox potential was measured with platinum electrodes and oxygen concentration with oxygen optodes, employing the dynamic quenching of luminescence as measurement principle.

Part I: Sampling of the foundation pile surrounding

Around a series of foundations with wooden piles in the Netherlands, Germany and Italy chemical sediment and sediment water composition as well as redox potential was measured. The chemical sediment composition was mainly governed by sediment type (sand, clay), whereas sediment water composition reflected the exposure to sea or freshwater and the redox status of the sampling location. In general sites were anoxic. Bacterial wood decay was detected at all sites but with different magnitude orders reflecting the different environments. Apparently, wood degrading bacteria can be active in a large number of different environments, i.e. have broad ecological amplitudes. When the physico-chemical properties of sedimental water from foundations in sandy sediments were compared with wood degradation levels, it was observed that decreasing total nitrogen concentration, but not that of phosphate, accompanied increasing bacterial wood decay.

Part II: Investigating chemical sediment conditions at differently bacterial decayed wooden pile foundations in Amsterdam.

Comparison of physico-chemical sediment conditions at two wooden pile foundation sites in Amsterdam, showing different decay intensities but where comparable sediment profiles were present. Redox potential, oxygen and ground water table measurements were conducted biweekly over a year. Sediment solution was sampled quarter yearly. Oxygen and redox measurements (average -200 mV) indicated oxygen free conditions at both sites with the exception of a very dry summer at the severe bacterial decayed site. This coincided with ground water table fluctuations, which indicates head of foundation exposure at the severely degraded site in very dry summers. Variation of sediment water composition was more intense at the severely degraded site with respect to calcium and sulphate concentration. Pile surrounding water exhibit significantly higher total nitrogen concentrations at the light bacterial decayed site 6 mg L^{-1} respectively 1.7 mg L^{-1} at the severe bacterial decayed site.

Part III: Studying bacterial wood decay under low oxygen conditions – results from microcosm experiments

In this study we set up microcosm experiments with pine sapwood and bacterial decayed wood sticks placed in waterlogged sediment to establish, to monitor and manipulate the bacterial wood decay process. The experiment was divided into three parts which altered the conditions accordingly to 1. different oxygen supplies (air, air + O2, N2, air + water circulation) to water overlaying the sediment, 2. different nitrogen (nitrate and ammonium), phosphate and sulphate sediment concentrations and 3. elevated glucose and sulphate sediment concentration. Microcosms were equipped with oxygen sensors (optodes) and CO_2, CH_4 and N_2O head space measurement devices.

1. Wood decay was microscopically detected, classified for low decay intensities and was found to have occurred in all treatments after 150 days. The fastest rate of decay had developed in 120 days and was most intense under air + water circulation treatment. Gas emissions and C-budget calculations did not show any reliable correlation with bacterial decay intensity. Oxygen concentrations in the sediment were only measurable in the air + water circulation treatment.

2. Sediment nitrogen and phosphate addition prevented bacterial decay in wood samples after 155 days but sediment pH was also affected by such additions. Gas emissions did not show any pattern related to bacterial wood decay. Wood surrounded by low sediment nitrogen concentrations was more likely to be bacterial decayed than wood in nitrogen rich sediments.

3. Sediment glucose and sulphate addition also prevented bacterial attack on other lignocellulosic test materials (kapok fibres) during the observation period of 28 days.

Bacterial wood decay is present under nearly all environmental conditions but the velocity of decay varies. There are presumably a multitude of bacteria creating the characteristic erosion bacteria decay which together have wide ecological amplitudes.

The presence of oxygen traces accelerates bacterial wood decay but does not seem to be a perquisite for its occurrence.

It was proposed that under nitrogen poor conditions wood is more susceptible to bacterial wood decay than under nutrient rich conditions, although this is only true for sandy sediment. For other sediment types being frequently surrounded by wooden pile foundations i.e. clay and peat separate investigations are needed.

Zusammenfassung

Historische Bauwerke an europäischen Küstengebieten und an Flussläufen sind häufig auf Fundamenten aus hölzernen Pfählen gegründet. Diese werden verwendet, um Bauten in Gegenden mit geringer Bodentragkraft zu stabilisieren. Viele einzigartige Baudenkmäler haben oder hatten Fundamente aus hölzernen Pfählen, zum Beispiel die Reichstage in Stockholm und Berlin, fast alle Gebäude im westlichen Teil der Niederlande wie in Amsterdam, Rotterdam und Haarlem sowie in Venedig, Italien. Diese Holzgründungskonstruktionen werden meist von Pilzbefall betroffen, wenn der Grundwasserspiegel soweit abgesenkt wird, dass Teile der Konstruktion frei liegen. Solange die Holzpfähle im wassergesättigten Sediment stehen, sehr geringe Sauerstoffkonzentrationen vorliegen oder sogar anoxische Bedingen herrschen, ist das Holz vor Pilzbefall geschützt. Allerdings können Bakterien das Holz besiedeln und ebenfalls zu seiner Zersetzung führen. Neuere Untersuchungen aus den Niederlanden und Schweden haben gezeigt, dass bakteriell befallenes Holz bereits in etwa hundert Jahren an Festigkeit verliert. Dabei sind die bisherigen Kenntnisse über die beteiligten Bakterienarten sowie deren Wachstumsbedingungen unzureichend.

Hauptziel der vorliegenden Untersuchung war die Charakterisierung der Umweltbedingungen, die den bakteriellen Holzbefall in Pfahlgründungskonstruktionen fördern. Im Einzelnen sollte geklärt werden: i.) welche Rolle gelöster Sauerstoff beim bakteriellen Holzbefall spielt ii.) ob erhöhte Stickstoffkonzentrationen in dem Medium, das das Holz umgibt, den bakteriellen Holzbefall begünstigen und iii.) ob Sulfatzugabe zum Sediment einen Schutz gegen eine Besiedelung des Holzes mit Bakterien darstellen würde. Um diese Fragen beantworten zu können, kamen drei verschiedene Untersuchungen zur Anwendung.

Aus Freilandbeprobungen und Laborversuchen wurden Sediment- und Sedimentwasserproben untersucht. In den Sedimentproben erfolgte eine Bestimmung von Gesamtkohlenstoff-, Gedamtstickstoff-, Schwefel- und Hauptkationen-Konzentration. In den Sedimentwasser-proben wurden der pH-Wert, die Leitfähigkeit, die Konzentrationen an Gesamtkohlenstoff und gelöstem Kohlenstoff, die Stickstoffkonzentrationen sowie die Konzentrationen von Ammonium, Nitrat, Sulfat, Phosphat, Chlor und Hauptkationen analysiert.

Teil 1: Probenahme in der Umgebung des Fundamentpfahles

Die chemische Zusammensetzung des Sedimentes und des Sedimentwassers zusammen mit dem Redoxpotential wurden in der Umgebung von Fundamenten mit hölzernen Pfählen in den Niederlanden, Deutschland und Italien gemessen. Die chemische Zusammensetzung des Sediments war vorwiegend durch die Art des Sedimentes bestimmt, während die Zusammensetzung des Sedimentwassers durch den unterschiedlich starken Einfluss von Meerwasser geprägt war. Generell lagen anoxische Bedingungen vor. Alle Probenahmestellen wiesen bakteriellen Holzbefall jedoch unterschiedlicher Intensität auf. Holzabbauende Bakterien können anscheinend unter zahlreichen Umweltbedingungen aktiv sein, das heißt sie haben eine weite ökologische Amplitude.

Teil II: Chemische Untersuchungen am Sediment verschiedener bakteriell befallener hölzerner Fundamentpfähle in Amsterdam.

An zwei unterschiedlich stark befallenen Holzfundamenten in Amsterdam mit vergleichbaren Sedimentprofilen erfolgte ein Vergleich der physiko-chemischen Sedimentwasser-eigenschaften. Über den Zeitraum eines Jahres wurden 2003/2004 zweiwöchentlich Messungen des Redoxpotentials, der Sauerstoffkonzentration und des Grundwasserspiegels durchgeführt. Sauerstoff- und Redoxpotential-Messungen (Mittelwert Eh -200 mV) deuteten auf sauerstofffreie Bedingungen an beiden Standorten hin. Eine Ausnahme trat im sehr trockenen Sommer 2003 an dem stärker befallenen Standort auf. Hier sank der Grundwasserspiegel so weit ab, dass der Kopf des Fundamentpfahles nicht mehr vom Wasser bedeckt war. Saisonale Veränderungen im Kalzium- und Sulfatgehalt des Sedimentwassers waren an dem stärker bakteriell befallenen Fundament deutlicher ausgeprägt. Im Vergleich mit dem stärker befallenen Standort zeigte das den Pfahl umgebende Wasser an dem weniger befallenen Standort eine signifikant höhere Gesamtstickstoffkonzentration auf.

Teil III: Untersuchungen zum bakteriellen Holzbefall unter geringen Sauerstoff-konzentrationen - Ergebnisse aus Mikrokosmen-Experimenten

Die Versuche fanden in Mikrokosmen statt, und zwar mit wassergesättigtem Sediment von einem stark befallenen Standort sowie intaktem und bakteriell befallenem Kiefernsplitholz, um einen Bakterienbefall zu etablieren, zu verfolgen und zu manipulieren. Dazu wurden drei verschiedene Experimente mit folgenden Versuchsbedingungen durchgeführt: 1.) Veränderung der Sauerstoffversorgung des Systems durch Begasung des das Sediment überlagernden Wassers mit Luft, Luft + Sauerstoff, Stickstoff sowie Luft + Wasserzirkulation.

2.) Veränderung der Stickstoff- (Ammonium und Nitrat), Phosphat- und Sulfatkonzentration im Sediment und 3.) Zugabe von Glukose und Sulfat zum Sediment. In Mikrokosmen wurde Sauerstoff mit Optoden sowie die CO_2-, N_2O- und CH_4-Produktion gemessen. Der Holzbefall wurde durch lichtmikroskopische Bestimmung klassifiziert.

1.) Nach 150 Tagen war bei allen Behandlungsformen das Holz befallen. In der Luft + Wasserzirkulations-Variante trat der Befall bereits nach 120 Tagen auf und war hier auch am stärksten ausgeprägt. Gasemissionen und Berechnungen der Kohlenstoffbilanz zeigten keine konstanten Relationen zur Intensität des bakteriellen Holzbefalls. Sauerstoffkonzentrationen größer Null wurden nur in der Variante Luft + Wasserzirkulation im Sediment gemessen.

2.) Bei einer Stickstoff- und Phosphatzugabe zum Sediment konnte in den Holzproben nach 155 Tagen kein bakterieller Befall festgestellt werden. Durch die Zugabe wurde allerdings der pH Wert verändert. Messungen der Gasemissionen ergaben keinen Zusammenhang zum bakteriellen Befall. Holz, welches von Sediment mit einem geringen Stickstoffgehalt umgeben ist, unterliegt einer höheren Wahrscheinlichkeit bakteriellen Befall aufzuweisen, verglichen mit Holz, welches von Sediment mit höherer Stickstoffkonzentration umgeben ist.

3.) Glukose- und Sulfatzugabe zum Sediment verhinderte bakteriellen Holzbefall an holzähnlichem Testmaterial (Kapokfasern) während des Untersuchungszeitraumes von 28 Tagen.

Bakterieller Holzbefall tritt unter sehr verschiedenen Umweltbedingen auf. Dabei variiert aber die Geschwindigkeit des Befalls. Es wird angenommen, dass eine Vielzahl von Bakterien den für Erosionsbakterien (erosion bacteria) typischen Befall hervorruft und dass diese eine große ökologische Amplitude aufweisen. Die Anwesenheit von sehr geringen Sauerstoff-konzentrationen beschleunigt den bakteriellen Befall, scheint jedoch keine Voraussetzung dafür zu sein. Es wird vermutet, dass Holz unter stickstoffarmen Bedingungen anfälliger für bakteriellen Holzbefall ist als unter eutrophierten Bedingungen. Diese Zusammenhänge wurden nur anhand sandiger Sedimenten gewonnen. Um zu klären in wieweit diese Ergebnisse auf andere hölzerne Fundamente umgebene Sedimente wie Ton und Torf übertragbar sind, besteht weiterer Forschungsbedarf.

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Abbreviations

A	Air (treatment in the MC experiment I)
A+C	Air and water circulation (treatment in the MC experiment I)
A+O_2	Air enriched with oxygen (MC experiment I)
AD	*anno Domini*, after Christ
Al	Aluminium
α	Level of significance
C	Carbon
C/A	Control (with out wood) of the air treatment (MC experiment I)
C/A+C	Control (with out wood) of the air and water circulation treatment (MC experiment I)
C/A+O_2	Control (with out wood) of the air enriched with oxygen (MC experiment I)
C/N	Control (with out wood) of the nitrogen treatment (MC experiment I)
Ca	Calcium
CFB	Cytophaga, Flavobacterium, Bacterioides complex
CH_4	Methane
Cl	Chloride
CO_2	Carbon dioxide
Ct	Total carbon
dl.	Detection limit
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
DW	Dry weight
EB	Erosion bacteria
Eh	Redox potential corrected for the standard hydrogen electrode [mV]
EU	European Union
Fe	Iron
FeS_2	Pyrite
GC	Gas chromatograph
HCO_3^-	Hydrogen carbonat
K	Potassium
LBD	Light bacterial decayed site

M	Mixed treatment from sand from Amsterdam and silica sand (MC experiment II)
MC	Microcosm
Mg	Magnesium
Mn	Manganese
N	Nitrogen
n	Number
n.a.	Not analysed
N_2	Nitrogen gas
N_2O	Nitrogen dioxide
Na	Sodium
NAP	Normal Amsterdam level
NH_4^+	Ammonium
NO_3^-	Nitrate
N_{org}	Organic nitrogen
N_t	Total soil nitrogen
OM	Organic matter
P	Phosphor
pe	Negative logarithm of the electron activity (pe = -lg [e]
PO_4^{3-}	Phosphate
P_t	Total phosphor
S	Sandy sediment from Amsterdam (MC experiment II)
S+A	Sediment from Amsterdam with ammonium addition (MC experiment II)
S+N	Sediment from Amsterdam with ammonium addition (MC experiment II)
S+P	Sediment from Amsterdam with phosphate addition (MC experiment II)
S+S	Sediment from Amsterdam with nitrate addition (MC experiment II)
SBD	Severe bacterial decayed site
SE	Standard error
SO_4^{2-}	Sulphate
SS	Silica sand
S_t	Total sulphur
TOC	Total organic carbon

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1 Introduction and state of the art

1.1 General introduction

Wooden pile foundations are widely used along coastal areas and river sites in Europe to support historic buildings. They are in service to stabilise constructions in areas with soils of low bearing capacity. As well as many outstanding monuments, such as the parliament building in Stockholm and the ‘Reichstag’ in Berlin, nearly all of the buildings of Amsterdam, Rotterdam, Haarlem (The Netherlands) and Venice (Italy) rest on foundations comprising wooden piles. When the ground water level is low these wooden structures are exposed to air and they can be attacked by fungi causing degradation, unless they are protected from oxygen contact (Boutelje and Bravery 1968; Paajanen und Viitanen 1988). Fungal degradation is prevented as long as the wooden constructions remain water logged, when oxygen availability is strongly reduced or anoxic conditions prevail. Under water logged conditions, however, bacteria can colonize the wood, leading to a certain degree of decay (Holt und Jones 1983). Until the 1970’s, bacterial wood decay was considered to be an extremely slow process, threatening only archaeological wooden specimens, such as shipwrecks, building remains or tools. Although bacterial wood degradation is slow compared to that cause by fungi, recent observations from The Netherlands and Sweden showed that bacterial activity under anoxic conditions can cause considerable strength loss of wooden foundations within a time span of one hundred years, endangering modern constructions (Boutelje and Göransson 1975; Klaassen 2007a).

To gain new insides in bacterial wood decay in wooden foundations and archaeological findings the European project BACPOLES (´Preserving cultural heritage by preventing bacterial decay of wood in foundation poles and archaeological sites´) was initiated 2002, involving microbiologist, wood and soil scientist, foundation engineers, archaeologist and conservators.

The main aims of the project were:

i. To characterise selected sampling sites throughout Europe on bacterial wood decay and its intensity and to link the data with chemical sediment and sediment water composition and redox potential and oxygen measurements.

ii. To investigate sampled wood from foundation piles and archaeological sites on type of decay and intensity, physical wood properties and chemical composition and to date the wood by year ring analysis.

iii. To isolate and identify wood degrading bacteria by applying DNA analysis to infer phylogenetic relationships based on comparative analysis with known sequences stored in databases.

iv. To conduct laboratory experiments to determine environmental conditions which favour bacterial wood decay.

v. To develop a possible preservation strategy against bacterial wood decay involving phages.

The project combined the experience from several European research institutions from five countries under the coordination of SHR Timber Research from Wageningen, The Netherlands. The microbiological investigations were conducted at the Swedish University of Agriculture, Department of Wood Sciences and at University of Portsmouth, School of Biological Sciences, UK. The sampling of the foundation piles and their surrounding was organised from Fugro Ingenieursbureau BV, Amsterdam, whereas for the archaeological sampling The National Service for Archaeological Heritage in Amersfoort, The Netherlands was responsible. From the Göttingen University two institutes worked closely together the Institute of Soil Science and Forest Nutrition and the Institute of Wood Biology and Technology.

1.2 Outline of the thesis

This thesis presents investigations which were conducted as part of the chemical characterisation of sediments and sediment water surrounding wooden foundations and archaeological remains of the BACPOLES project. In Chapter 2 the objectives of this thesis are stated followed by chapter 3 where the methods used are explained. The results and discussion section is composed of three parts, in which the main research topics are addressed. Chapter 4 describes sediment conditions around wooden foundation piles in The Netherlands, Italy and Germany and relates them to bacterial wood decay. Chapter 5 presents the comparison of sediment conditions surrounding two differently bacterial decayed wooden

foundations in Amsterdam with similar sandy sediment. Chapter 6 presents laboratory microcosm experiments with water logged sandy sediment in which infected and sound wood samples were exposed to different gradients of oxygen availability and chemical sediment compositions.

The microcosm experiment (Chapter 6) was conducted as common project with Jana Gelbrich from the Institute of Wood Biology and Technology of the Göttingen University, who investigated the wood properties before and after incubation. The microscopic decay detection was her work and will be presented in-depths in her PhD thesis (Gelbrich 2008, in preparation). The results of the bacterial decay detection are included in this thesis to aid the interpretation of the chemical results. The parts are clearly marked and cited as parts from the conjointly written paper.

Finally the findings from chapter 4-6 are discussed together in chapter 7 in the attempt to enlighten environmental conditions favouring bacterial wood decay and conclusions are drawn from the presented work.

1.3 Wood decay

Wood decay is a complex process involving insects, fungi and bacteria (Eaton and Hale 1993). Insects, which degrade wood, include marine borers, termites, boring beetles and ants or wasps (Zabel and Morrell 1992). Except for the marine borers insects do not degrade water logged wood. Marine wood-boring animals destroy wood over a short period in oxygenated marine environments. In temperate climate, these animals are predominantly the shipworms (Teredinidae) and gribble (Limnoriidae) (Eaton and Hale 1993). Depending on the environment, different types of microorganisms dominate active wood degradation (Kaarik 1974). The most destructive microorganisms are white-rot and brown-rot fungi which are classified as basidiomycete fungi. However, they are only active in wood that is partly dried out (20 - 80%), and which has an ample supply of oxygen. If the wood is water saturated but oxygen is still present soft-rot fungi belonging to the ascomycete and fungi imperfecti groups are the main wood degraders. All these fungi will destroy the wood completely in relatively short times. However, when the presence of fungi is precluded by very limited oxygen availability under water logged conditions bacteria are the main wood degraders.

1.4 Wood degrading bacteria

Based on their specific morphological features three types of wood degrading bacteria are distinguished as cavity, tunnelling and erosion bacteria (Daniel and Nilsson 1986; Blanchette et al. 1990; Singh and Butcher 1991). They are slower wood degraders than fungi. All bacteria are described as rod-shaped but they are not identified yet (Daniel and Nilsson 1997, Landy et al. 2005). However, there is evidence that erosion bacteria belong to the Cytophaga, Flavobacterium, Bacterioides (CFB) complex (Landy 2007). Under waterlogged conditions with very restricted oxygen supply erosion bacteria (EB) were found to be the main wood degraders (Singh and Kim 1997). Increasing evidence exists to suggest, that erosion bacteria may be most tolerant to near-anoxic conditions (Singh et al. 1990; Blanchette and Hoffman 1993; Kim and Singh 1994; Singh et al. 1994). Nevertheless, the occurrence of lignin degradation under completely anoxic conditions could so far not be proved without doubt (Björdal 2000).

Wood degradation by erosion bacteria starts from the surface proceeding inwards. Rays have been observed to be preferred pathways in softwoods, where cross field pits provide assess to the axial tracheids. Erosion bacteria are able to glide, which facilitates the movement within the wood (Björdal and Nilsson 2005).

In a study comparing bacterial wood decay in a natural lake with laboratory trials, applying isolated bacteria, Schmidt et al. (1987) found bacterial wood decay only in the lake samples after twelve months. Attempts to obtain degradation of lignified wood components using pure cultures mostly failed (Jones et al. 1986; Schmidt und Liese 1994) and only one study reported success with *Aureobacterium luteolum* on 20 µm thin pine sections (Schmidt et al. 1995). Therefore, it was hypothesised that bacterial communities living in symbiosis are responsible for wood decay (Daniel and Nilsson 1986; Nagashima et al. 1988). Nevertheless, applying mixed cultures derived from decayed wood from aquatic or terrestrial environments, bacterial wood decay can be initiated in laboratory settings (Daniel and Nilsson 1986; Singh 1989; Singh et al. 1990). Studies under controlled conditions investigating environmental factors influencing bacterial wood decay are rare.

1.5 Environmental factors influencing bacterial wood decay

Little is known about the specific environment in which bacterial wood decay proceeds, as the culturing of wood degrading bacteria failed so far. Only Boutelje and Göransson (1975) investigated the surrounding sediments and ground water of wooden foundations chemically and related it to wood decay. They proposed elevated sediment nitrogen and phosphate concentrations in the surrounding of the piles being responsible for pile damage found in Stockholm. Other available studies on wood decay in foundation piles only comment on the surrounding sediment type (Paajanen and Viitanen 1988; Grinda 1997). Nevertheless, information on environmental factors influencing the survival of waterlogged wood in archaeological sites is available (Caple 1994) and was reviewed by Jordan (2001). He stated that dissolved oxygen, pH, redox potential (Eh), and the presence of certain ion species as nitrate or ammonium are the most common reported characteristics of depositional environments.

1.5.1 Oxygen

The dissolved oxygen concentration is a key parameter when investigating wood decay, because wood degrading fungi rely on oxygen based enzymes. Soft rot fungi are most tolerant to a diminished oxygen availability of up to 0.48 mg L^{-1} (Kohlmeyer and Kohlmeyer 1979). When oxygen concentration is low, bacteria are the main wood degraders. Up to now it is not unambiguous proved whether erosion bacteria (EB), the type of bacteria most tolerant to depleted oxygen concentrations (Björdal et al. 2000), are active in anoxic conditions (Blanchette et al. 1990; Nilsson 1999). Björdal et al. (2000) found a depth gradient in the degree of bacterial wood degradation in archaeological poles founded in river sediments and related this finding to small differences in oxygen concentration derived from different depth of burial in the ground. However, most of the papers available on bacterial wood decay judge oxygen concentration as being one of the mayor environmental factors favouring bacterial wood decay but did not conduct oxygen measurements (Singh et al. 1990; Blanchette and Hoffman 1993; Björdal et al. 1999).

In the literature on bacterial decay in archaeological wood (Caple 1994; Jordan 2001) no clear differentiation is made between anoxic and anaerob. In this work anaerob denotes the

metabolism without oxygen as terminal electron acceptor, whereas anoxic denotes the oxygen free environment.

1.5.2 pH

The majority of bacteria grow best under pH 7 but a variety of species tolerate slightly alkaline conditions (Schlegel 1992). However, subgroups may have extreme optima i.e. sulphur oxidisers are able to grow at pH's as low as 2 (Eaton 2005), whereas sulphur reducing bacteria only function above pH 5.5 (Cronyn 1992). It is assumed that wood-degrading bacteria have a comparable optimum as bacteria in general but con tolerate a wide range of pH values, with tunnelling bacteria are preferring more alkaline conditions (Nilsson 1999; Jordan 2001). The pH value of wood is about 4 to 6.5 (Schmidt and Liese 1994). However, long-term storage in sediment with or without water logging will influence wood pH towards sediment water pH. In most water logged environments a neutral to slightly alkaline pH prevails (Caple 1994; Jordan 2001). The redox status of an environment influences pH as occurring reactions liberate or consume protons (H^+).

1.5.3 Redox Potential

The word redox is a composite formed from reduction which is a gain of electrons and oxidation a loss of electrons (Bartlett 1998). Negative values are defined as anoxic environments which have a surplus of electrons, whereas positive values denote oxic conditions with an electron shortage (Sigg 2000). The Nernst equation (Eq. 1.1) describes the inverse relationship between Eh and pH, where E^0 is the standard potential, R = gas constant, T = temperature in K, n = number of electrons involved in the reaction, F = Faraday constant, a_{Ox} = activity of the oxidised compound, a_{Red} = activity of the reduction partner (Appelo and Postma 2005).

$$Eh = E^0 + \frac{RT}{nF} \ln \frac{a_{Ox}}{a_{Red}} \qquad \text{Eq. 1.1}$$

Any change in redox potential is mirrored in a pH change and vice versa (Ponnamperuma 1972). Comparable to the pH as the hydrogen ion activity is p*e* defined as electron activity (e^-) (Eq. 1.2) which is related to Eh has shown in Eq. 1.3.

$$pe = -\lg(e^-) \qquad \text{Eq. 1.2}$$

$$pe = \frac{Eh}{2{,}3RT/F} \qquad \text{Eq. 1.3}$$

Field measurements can be visualised in a p*e* – pH diagram (Fig. 1.1). In this diagram the stability of water marks the region of possible redox and pH values, whereas the area of p*e* and pH values occurring in natural waters is smaller.

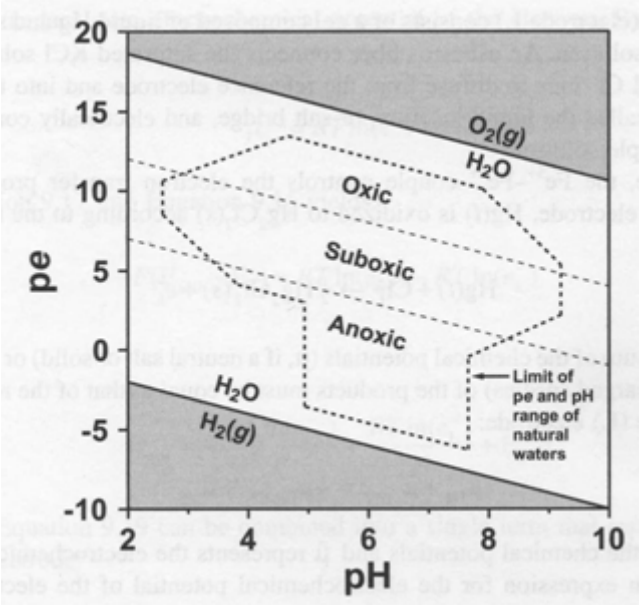

Fig. 1.1: The pe – pH stability region of water with the measured limits of redox zones in natural water and the redox zones of Sposito (1989) taken from Essington 2004.

Several empirical ranges of Eh can be derived where different redox systems are active and support bacterial metabolism (Tab. 1.1). According to the thermodynamic theory these reactions are sequential. However, field observations find often more than one reduced species or reduction indicator concurrently present which suggest, that different reductions are

occurring at the same time (McBride 1994). This can be attributed to the heterogenic distribution of organic matter and oxygen in aggregates and a lack of equilibrium (Bohn 1970). The redox potentials for the different redox reactions can only be given with wide amplitudes as additionally to the above-mentioned heterogeneity the stability of oxides, i.e. iron or manganese, does influence which chemical reaction is energetically favoured (Peiffer 2000). Postma and Jakobsen (1996) could demonstrate that the division of the reductions of the various electron acceptors into different redox zones is better understood as partial equilibrium process.

Tab. 1.1: Different redox reactions and their experimentally deduced redox potentials (Schachtschabel et al. 1992) together with redox zones proposed by Sposito (1989)

Reaction	Redox Indicator	Redox Potential [mV]	Redox zone *
Aerobic respiration	Oxygen	800 - 450	Oxidised
Denitrification	Nitrate	700 - 450	(Eh > 414 mV)
Manganese reduction	Manganese (II)	450 - 350	Suboxic
No oxygen detectable		< 330	(Eh 414 – 120 mV)
No nitrate detectable		< 220	
Iron reduction	Iron (II)	< 150	Anoxic
Sulphate reduction	Sulphate	< 0	(Eh < 120 mV)
Methane Production	Methane	< -120	
No Sulphate detectable		< -180	

* According to Sposito (1989)

The theory of redox potential measurements is based on thermodynamic considerations. The current state of a chemical equilibrium of a chemical reaction which is reversible and includes electron transfers can be described by the potential of an inert (platinum) electrode (Schüring et al. 2000). If there is more than one chemical reaction occurring at the same time a mixed potential is measured which is determined by the redox couples with the highest exchange currents. It is necessary that the existing redox reactions can be catalysed by the electrode material (platinum). If not - as it is case for methane, nitrogen gas (N_2) and stable humic

substances bound to a solid phase – the reaction does not contribute to the measured redox potential (Bartlett 1998). This is to a lesser extent also the case for nitrate, bicarbonate and sulphate. Furthermore, measurements represent only the redox status of the dominant redox couple if the electron transfer is fast enough (Sigg 2000). Redox measurements are difficult to interpret because it is not clear which sediment the electrode measurements represent as the electrode position with respect to large pores is unknown. Platinum forms stable oxides with oxygen (Sigg 2000). Oxygen containing structures in sediments are, therefore, over represented compared to sediment structures with other redox systems, which react on a slower rate with the electrode material. Thus, in aerobic soil, platinum electrodes are not a reliable indicator of redox status (Esslington 2004). However, most of the problems concerning redox potentials appear if redox measurements are over interpreted towards thermodynamic equilibria (Sigg 2000). Therefore, the combination from oxygen measurements in oxic conditions with redox potential measurements in sub oxic and anoxic conditions provides additional information.

1.5.4 Nutritional composition

Bacteria need nitrogen for protein synthesis. A mean cell consists of approximately 14 % nitrogen, which must be taken up by the bacteria (Schlegel 1992). This is the function of nitrogen as nutrient for bacterial growth. However, under low oxygen condition facultative anaerob bacteria are able to use nitrate instead of oxygen as terminal electron acceptor for energy generation. Soil microorganisms are known to be nitrogen and phosphate restricted (Schachtschabel et al. 1992). The availability of other nutrients as nitrogen could regulate, additionally, bacterial wood decay activity.

1.6 Additional factors influencing bacterial wood decay intensity

Wood from deciduous trees is less susceptible to bacterial decay than wood from coniferous trees (Daniel and Nilsson 1997). Sapwood is much more susceptible to degradation than heartwood (Grinda 1997; Klaassen 2007a). This is related to the presence of toxic extractives in the heartwood. Furthermore pine is more susceptible to bacterial decay compared to spruce

(Klaassen 2007a). It is proposed that the open structure of pine sapwood promotes water flow which provides nutrients to erosion bacteria and fosters their growth (Klaassen 2007b).

1.7 Oxygen measurements

Normally oxygen is measured with a silver platinum (Clark) electrode which consumes oxygen. Therefore, a constant stream of the liquid sample is either archived by stirring a probe or measuring in a liquid stream. The electrodes are often sensible to stirring velocity. To measure low oxygen concentrations or in small sample sizes microelectrodes are used, which consume little oxygen amounts and have delicate dimensions. The handling of these electrodes especially in the field is difficult as they break easily and are expensive. Fiber-optic sensors (called optodes) based on dynamic fluorescence quenching provide a possibility to measure oxygen without oxygen consumption and stirring dependency (Klimat et al. 1995). For oxygen measurements in sediments it is necessary to place the sensor without air oxygen contamination. As the device does not consume oxygen, introduced air oxygen will be measured until consumed by oxidation of reduced elements or aerobic respiration.

2 Objectives

In view of the above state of the art, the primary objective of this dissertation was to investigate under which environmental conditions bacterial wood decay exists in foundation piles. In detail the aims were:

1. To characterise the environmental conditions which promote bacterial wood decay.

    – To describe the seasonal variations in the sediment and sediment water composition.

2. To examine if the presence of oxygen concentration is a prerequisite for bacterial wood decay.

    – To study if very low redox potentials can prevent bacterial wood decay

3. To elucidate if elevated nitrogen concentrations (due to eutrophication) in the wood surroundings favour bacterial wood decay.

    – To investigate if phosphate addition to sediment surrounding wood promotes bacterial wood decay

4. To determine if CO_2 can serve as alternative bacterial wood decay detection method in a laboratory experiment

The work consists of three different parts which are related to each other as shown in Fig. 2.1.

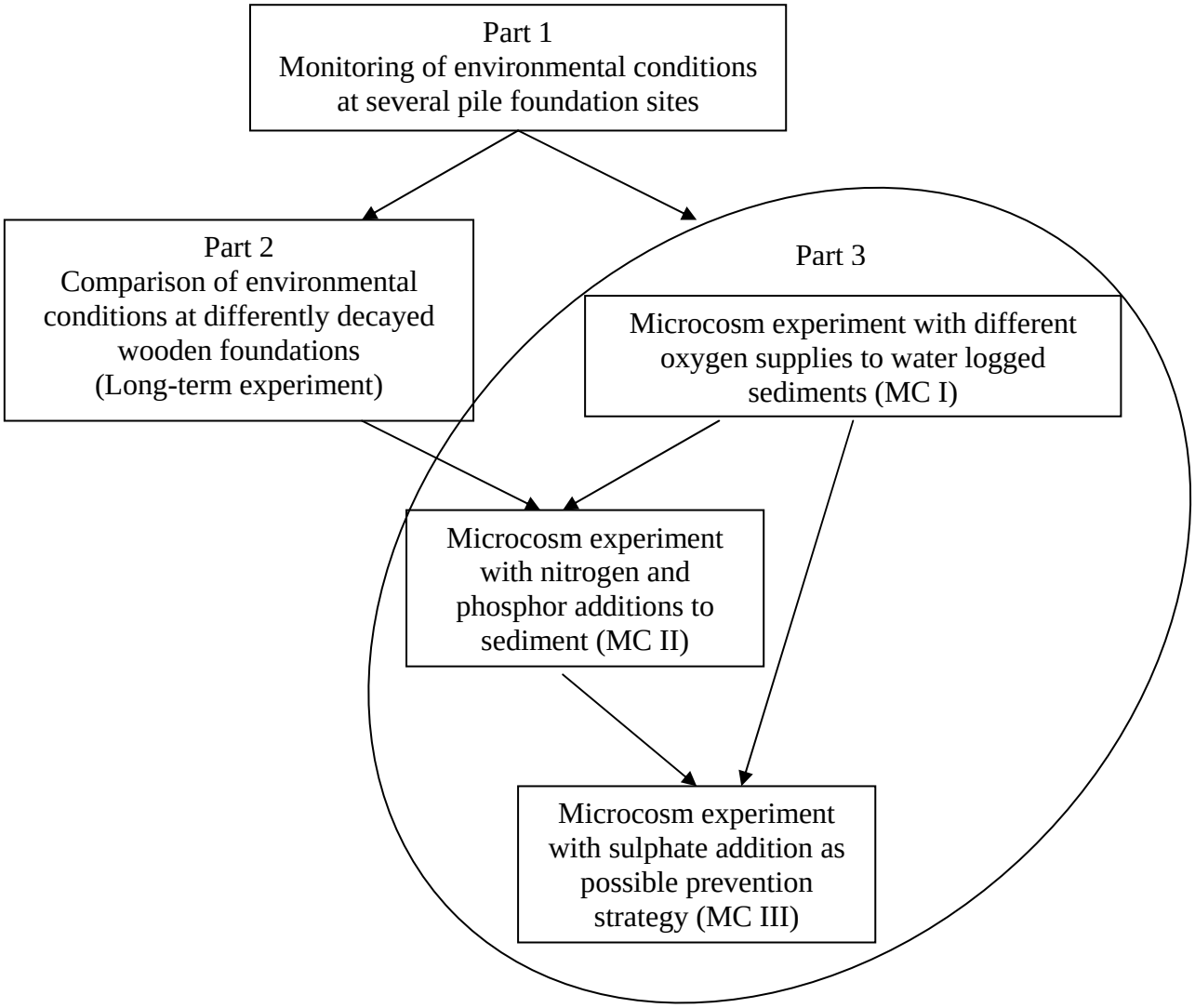

Fig. 2.1: Relation of the three parts of the work to each other, (MC = microcosm)

Part 1: Environmental conditions which favour bacterial wood decay were monitored. Thus, physico-chemical sediment conditions under which bacterial wood decay occurs in foundation piles in The Netherlands, Italy and Germany were investigated. Furthermore, it was elucidated, whether eutrophication especially high nitrogen concentrations of the pile surroundings promotes bacterial wood decay.

Part 2: Oxygen and redox potential measurements were combined with chemical sediment characteristics and were related to bacterial wood decay by comparing a severely and a little bacterial decayed wooden pile foundation site with comparable sediment profiles

in Amsterdam, The Netherlands. Additionally, seasonal variations of physico-chemical parameters at the two sites were studied to aid interpretation of single measurements at wooden pile foundations.

Part 3: A microcosm experiment was conducted to confine the gap between field experiments with no records of prevailing physico-chemical factors and controlled laboratory experiments with good documentation of the governing factors. The microcosms consisted of wood buried in waterlogged sediment, aimed to establish bacterial wood decay with naturally occurring bacteria and measurements of free dissolved oxygen. Three experiments were conducted:

3.1. To investigate the role of oxygen in the bacterial decay process the system was subject to different gassing treatments to the water overlaying the sediment.

- Additionally it was investigated whether other parameters as light microscopic decay detection could serve as indicator for bacterial wood decay under laboratory conditions. Therefore, CO_2, N_2O and CH_4 gas productions in the microcosms were monitored

3.2. The goal of this experiment was to investigate if the decay process is influenced by the chemical sediment composition. This was accomplished by adding nitrogen either as nitrate or ammonium and phosphate and sulphate to the water saturated sandy sediment surrounding infected and sound wood samples.

3.3. The third experiment was intended to test a simple preservation strategy against bacterial wood decay. Based on the outcome of the first two microcosm experiments easy degradable organic matter in the form of glucose was added to the sediment to deplete oxygen and provide an alternative food source for microorganisms. In order to further lower the redox potential sulphate was additionally added. The experiment was conducted with kapok, a well established surrogate material for wood, which shows much faster signs of bacterial decay than wood.

3. Material and methods

3.1. Sampling of foundation pile surroundings

3.1.1. Sampling sites

The sites used in this study were selected to represent various sediment conditions that exist around wooden foundations. At some sampling locations the wooden foundation piles were removed which enabled the investigation of the whole pile length. An overview of the sites sampled together with sediment type, foundation age and wood species is pesented in Tab. 3.1. The sites located in The Netherlands are shown in a map (Fig. 3.1).

Two sites in Venice ("Venice bridge" and "Venice house") are on either side of the San Marco canal in Venice, here the foundations are surrounded by fine, organic rich lagoon deposits (Abrami 2003). The site in Amsterdam was located in the Spaarndammerbuurt and consists of buildings, 4 or 5 stories high, that were erected in 1918 (Keijer 2003a). The foundations were in sandy sediment over Holocene peat resting on a Pleistocene sand layer. The sampling in Dordrecht, Paulownastraat was done on a house which is part of a block of houses (erected during 1931), consisting of 6 attached houses with each 2 stories and an attic (Keijer 2003b). The piles resided in loamy sediment over peat. In Haarlem complete piles were removed from two blocks of attached house after they were demolished. They were built in 1904 (Dyserinckstraat) and 1895 (Vooruitgangstraat) and consisted of 2 stories. The foundation of these houses can be described as typical “Rotterdamse fundering”; where a single row of wooden piles are laid, on top of which longitudinal wooden beams are placed to support the masonry of the houses. Unfortunately the pile extraction disturbed the sediment at the site so much that sampling was done at a nearby house where the sediment was intact. The sediment consisted of a sand layer over a peat seam (Keijer 2005c). In Rotterdam the site was located at the corner of Joubertstraat and Paul Krugerstraat where a complete block of houses, erected between 1901 and 1905, had been demolished between these streets and the Bloemfonteinstraat. The piles supporting these buildings had an age of approximately 100 years. No construction drawings depicting the original foundation were available because they were destroyed during World War II when the Rotterdam government archives were obliterated. Nevertheless, a typical “Rotterdamse fundering” was expected, comprising a single row of piles mounted with longitudinal beams. The pile head level was measured at

1.88 m NAP (New Amsterdam level) and the piles were burried in clay sediment that covered a peat layer (Keijer 2003c).

Tab. 3.1: Foundation pile sites investigated together with building type, which part of the foundation was sampled, foundation pole wood species, felling date of the foundation piles, building age and foundation surrounding sediment

Sampling site	Foundation piles of:	Part of pile sampled	Wood species	Felling date of wood *	Building age [Years]	Sediment/soil type
Amsterdam	House	Pile head	Pine / Spruce	No date ~ 39-58 a	100	Sand
Dordrecht	House	Pile head	Spruce / Pine	1929 AD	100	Clay/sand
Haarlem	House	Pile extraction	Pine	1894-1904 AD	100	Sand over peat
Rotterdam	House	Pile extraction	Spruce / Fir	No Date ~ 53-98 a	100	Clay over peat
Zaandam	House	Pile extraction	Pine	1935 AD	100	Sand over peat
Travenhorst	Late medieval castle	Pile extraction	Oak / Spruce	No Date	650 #	Peat over sand
Borssele	Roman house	Pile extraction	Oak	100 AD	2000 #	Peat
Venice, Bridge	Bridge	Pile head	Oak / Fir / Larch	No Date	500	Anthropo-genic soil, clay/sand
Venice, House	Palacio	Pile head	Oak / Pine	No Date	500	Anthropo-genic soil, clay/sand

** Age of wood was determined by year ring analysis for details see Sass-Klaassen et al. 2007*

 # Building age is the age of the wooden piles as the sites are archaeological excavations. The buildings are not existent any more. In the case of Borssele the piles were part of the house structure and not part of the foundation, whereas in Travenhorst the piles were the original foundation poles.

The Zaandam site was located along the side facade of the house Irisstraat 89, Koog a/d Zaan, The Netherlands. The foundation for this building had to be underpinned in 2002 because its

bearing capacity had been reduced. It was possible, therefore, to extract the existing piles, as they no longer had a function. The house was built in 1937 and consists of 2 stories and an attic. The foundation of the house can be described as an “Amsterdamse fundering”; consisting of a double row of wooden piles above which a short horizontal wooden beam, of 40 mm thickness, was placed. Longitudinal wooden beams are placed above the horizontal ones and are used to support the masonry of the house. The piles were burried in sand covering a layer of peat (Keijer 2003d).

Two archaeological sites for study are also included in this report: One was located in Borssele, The Netherlands, and consisted of farmhouses with piles dated from Roman times, which were burried in an area of peat with marine influence (Siers 2002); the other was a medieval castle constructed around 1350 by the river Trave at Travenhorst, which is north east of Bad Segeberg in Schlesweig-Holstein, north Germany.

Fig. 3.1: Location of sampling sites in The Netherlands.

The piles were extracted from the foundation of this castle, which is situated in a wet area formed by the river. All of the piles were made from oak and rested in 1.0 to 1.1 m peat,

extending down to a sand layer underneath. The upper part of the piles had been exposed to air for two years and had deteriorated prior to sampling, but 10 cm under the dried material the peat and wood were still wet.

3.1.2. Field work and sampling

Foundations at all sites were inspected prior to sampling. This involved excavation to expose the foundations and pumping to remove water. Once the foundations were exposed, their depth, the wood species used to manufacture the piles, sediment layers and the environment surrounding the piles could be recorded. In the Netherlands information on the foundation depth, wood species used and the sediment layers were available in some cases. When this data was available, the depth needed for sampling could be pre-determined allowing the removal of sediment solutions and the recording of redox measurements to be made during excavation. The following sampling regime was used when the foundation was unexposed prior to excavation and the foundation depth was known: One sample was taken 50 cm above the pile head; another was made at the pile head; and the last was recorded 50 cm below the pile head. When the sediment layers above the pile head were previously removed, such as in the case of archaeological sites or where buildings had been demolished, samples were taken at the pile head followed by ones removed two depths below this level.

Sediment solutions were sampled using mobile lysimeter probes, with a ceramic suction lysimeter head (Bredemeier et al. 1990). Three water samples were taken, when possible, at +50, 0 and -50 cm relative to the top of the wooden pile. They were stored at 4°C in using a portable refrigerator. The pH of water sub-samples taken in the field were measured directly using a pH meter with glass electrode and a standard pH/conductivity meter (WTW GmbH, Weilheim, Germany). The redox potential was measured at fixed depths using the same standard pH/conductivity meter connected to a probe with four platinum electrodes, with a Ag/AgCl electrode used as a reference. The redox probe was arranged to reach sediment depths of approximately 20 cm above, and 25, 35, and 50 cm below, the top of the pile foundation or the top of the wood samples.

During excavation, three soil samples were taken at consecutive depths. Additionally, when possible, samples were taken from the groundwater around the wood that was released during excavation (referred to as collected surface water). These samples were stored at a

temperature of 4 °C and kept in the dark. Because the fieldwork had to be performed at sites where foundation inspection or archaeological excavations were ongoing, sampling and measurement plans were often adapted at the time of the work so as not to hamper the engineering or archaeological work.

Fig. 3.2 gives an overview of the redox measurements depths and depth where soil samples and water samples were taken relative to the top of the piles.

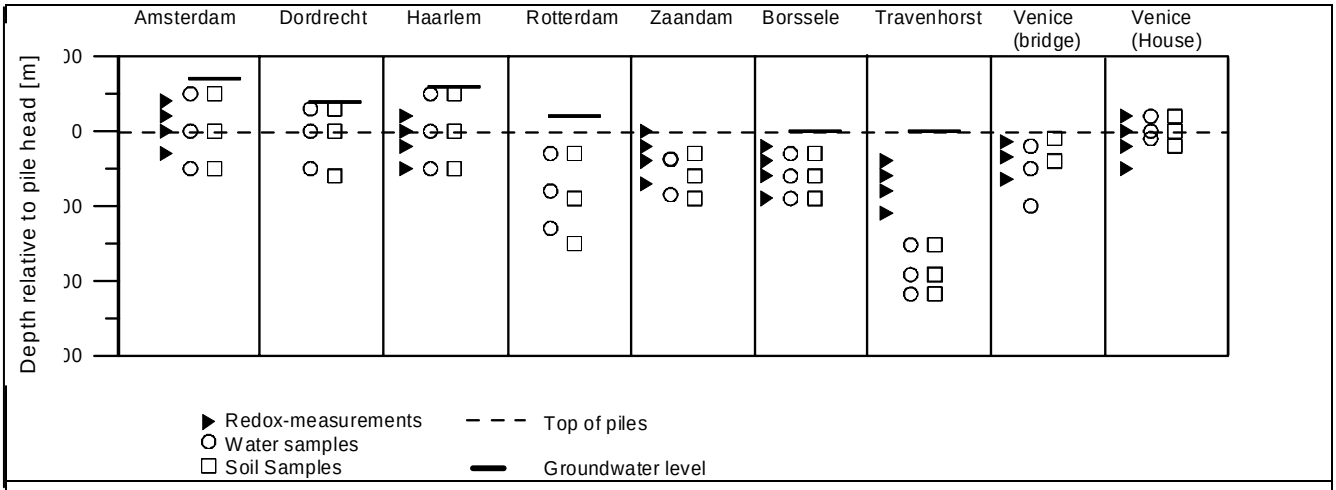

Fig. 3.2: Overview over ground water level, head of pile depth and sediment and sediment water sample depths together with redox potential measurement depths per site.

3.2. Long-term measurements

3.2.1. Measurement sites

Two locations were chosen from a survey of 33 foundation inspections in the city-quarter “De Pijp” in Amsterdam near the north-south Metroline (Fugro 2003). The Ferdinand Bolstraat was selected because it is situated in an area with comparable sediment profiles. This neighbourhood of Amsterdam, between the Stadhouderskade (north) and the Ceintuurbaan (south), was developed between the years 1875 and 1900. In the whole area the top layer consisted originally of a peat sediment. To obtain a dry top-layer of 1.0 m, about 3.0 meters of sand were deposited on top of the peat. One location is situated in the northern part (Fig. 3.3), where little bacterial decay was found in wooden foundation piles supporting buildings, whereas the other location in the southern part is located in an area with foundations severely decayed by bacteria.

Light bacterial decay site (LBD): located in the northern part of the Ferdinand Bolstraat 10 at the corner with 1^{e} Jacob van Campenstraat. The area was developed between 1875 and 1885/1890. Each pile head is approximately 1.2 meter under the average ground water level

Severe bacterial decay site (SBD): located in the southern part of Ferdinand Bolstraat near the Sarphatipark in the 1^{e} Jan van der Heijdenstraat 129. The area was developed between 1885/1890 and 1900. Each pile head is approximately 0.3 metre under the average ground water level.

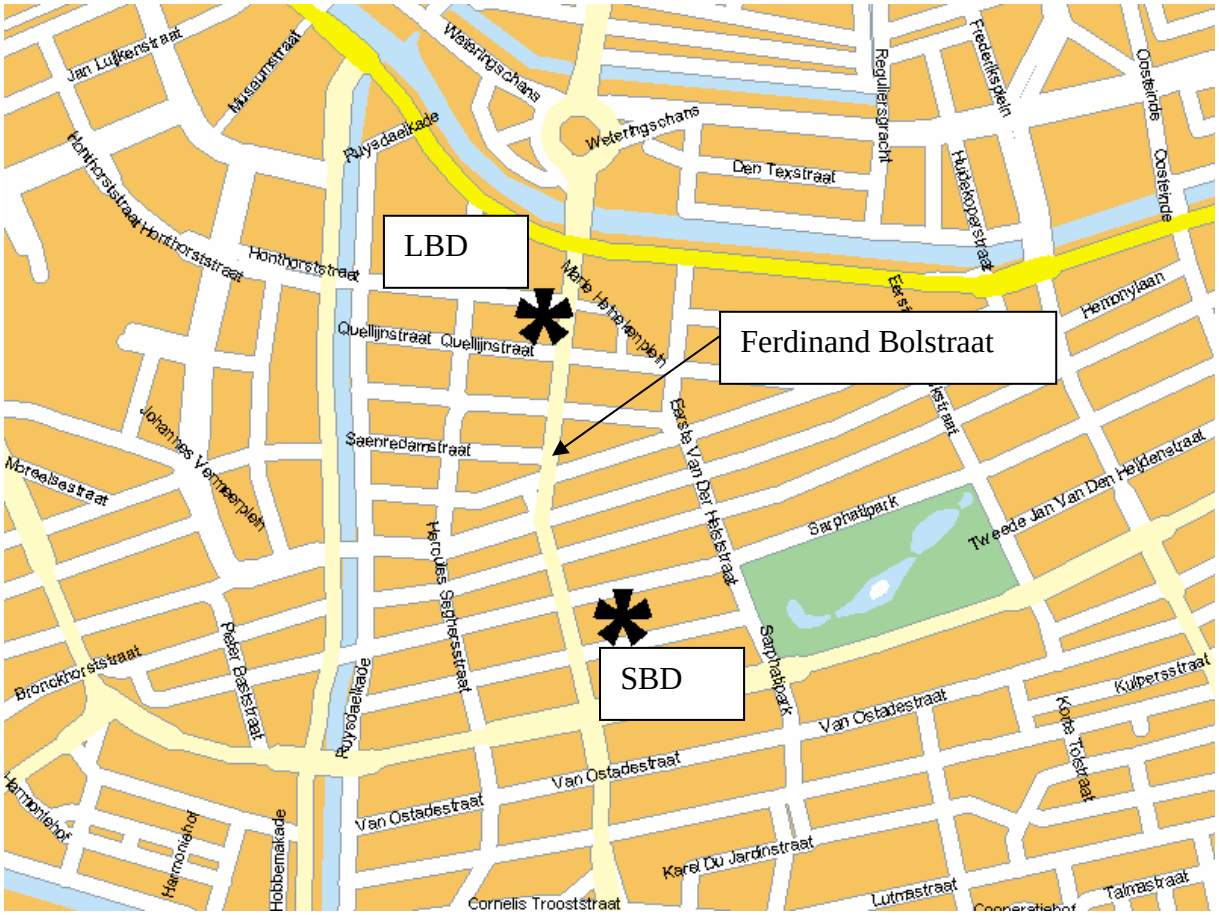

Fig. 3.3: Location of sites in Amsterdam, LBD: light bacterial decay located in the northern part of Ferdinand Bolstraat and SBD: severe bacterial decay situated in the southern part.

3.2.2. Measurements

At each site the oxygen concentration, redox potential, temperature and the ground water level were measured at different depths. The measuring points were arranged around the head of the foundation pile, for the installation depths see Tab. 3.2.

Tab. 3.2: Depths [m] of sensor installations in relation to pile head and absolute depth. Pile head is -1.16 m NAP (Normal Amsterdam Level) at LBD site and -0.61 m NAP at SBD site.

Light bacterial decay (LBD)				**Severe bacterial decay (SBD)**			
Depth [m NAP]	Depth in relation to pile head [m]			Depth [m NAP]	Depth in relation to pile head [m]		
	Oxygen	Redox Potential	Soil Solution		Oxygen	Redox Potential	Soil Solution
-0.66	+0.50	+0.50	+0.50	-0.31	+0.30	+0.30	+0.30
-0.86	+0.30#			-0.61	0.00		
-1.21	-0.05*	-0.05	-0.05	-0.66	-0.05*#	-0.05	-0.05
-1.26	-0.10			-0.71	-0.10		
-1.31	-0.15	-0.15		-0.76	-0.15	-0.15	-0.15
-1.46	-0.30			-0.91	-0.30		
-1.66	-0.50	-0.50	-0.50	-1.11	-0.50	-0.50	-0.50

*triplicate measurements, # temperature sensor

3.2.2.1. Oxygen and temperature measurements

The oxygen concentration was measured with oxygen optodes (PreSense, Regensburg, Germany). The Optode principle is the dynamic quenching of luminescence measuring the luminescence lifetime of a luminophore immobilised in a sensor foil (Klimant et al. 1995). For measurements the optodes are manually connected to the measurement device (Fibox 2-AOT). Before installation, the optodes were calibrated using water saturated air as 100% and water saturated N_2 as 0%. Long-term stability was checked by re-calibrating a spare optode kept under the same conditions. The temperature was measured at one depth only, with a temperature sensor.

3.2.2.2. Redox potential

The redox potential was measured using a probe with 4 platinum electrodes at fixed depths and a standard pH/conductivity meter (WTW GmbH, Weilheim, Germany). As a reference an Ag/AgCl electrode was applied. The redox probe was constructed with four measurement slots 0.2 m (firth three) and 0.3 m (forth one) apart from each other. The measured potential was corrected for the hydrogen electrode by adding 220 mV.

3.2.2.3. Ground water level, flow and direction

The ground water level was measured in a stand pipe (installed according to Dutch regulation NEN5120), which consists of a plastic pipe with rectangular vertical screenings and a fiber protection glove to prevent clogging of the openings by sediment particles at the lower end. The hole around the standpipe was filled with gravel to ensure water flow contact with the surrounding sediment. The height of the water table was measured by a tape measure equipped with a water sensitive tip (Eijkelkamp at Giesbeek, The Netherlands) which gives an acoustic signal on water contact.

Ground water flow and ground water flow directions were measured with a 40 GeoFlo® Model, (KV Associales, Inc.) using the heat-pulse principle.

3.2.2.4. Installation of measuring equipment

Fig. 3.4 shows the installation of the measurement equipment. Steel boxes with a dimension of 1.0 by 0.7 m were installed at both locations under the pavement. The steel cover of the boxes lay at the level of the pavement but the ground for installation of the boxes was excavated until approximately 0.6 m below the pavement. The location of the boxes was chosen to be as close as possible to the wooden foundation of the house. However, the horizontal distance between steel box and wooden foundation is about 2.2 m at the LBD site and about 3.5 m at the SBD site. Into the sediment under the open steel box all the oxygen optodes and other sensors were installed.

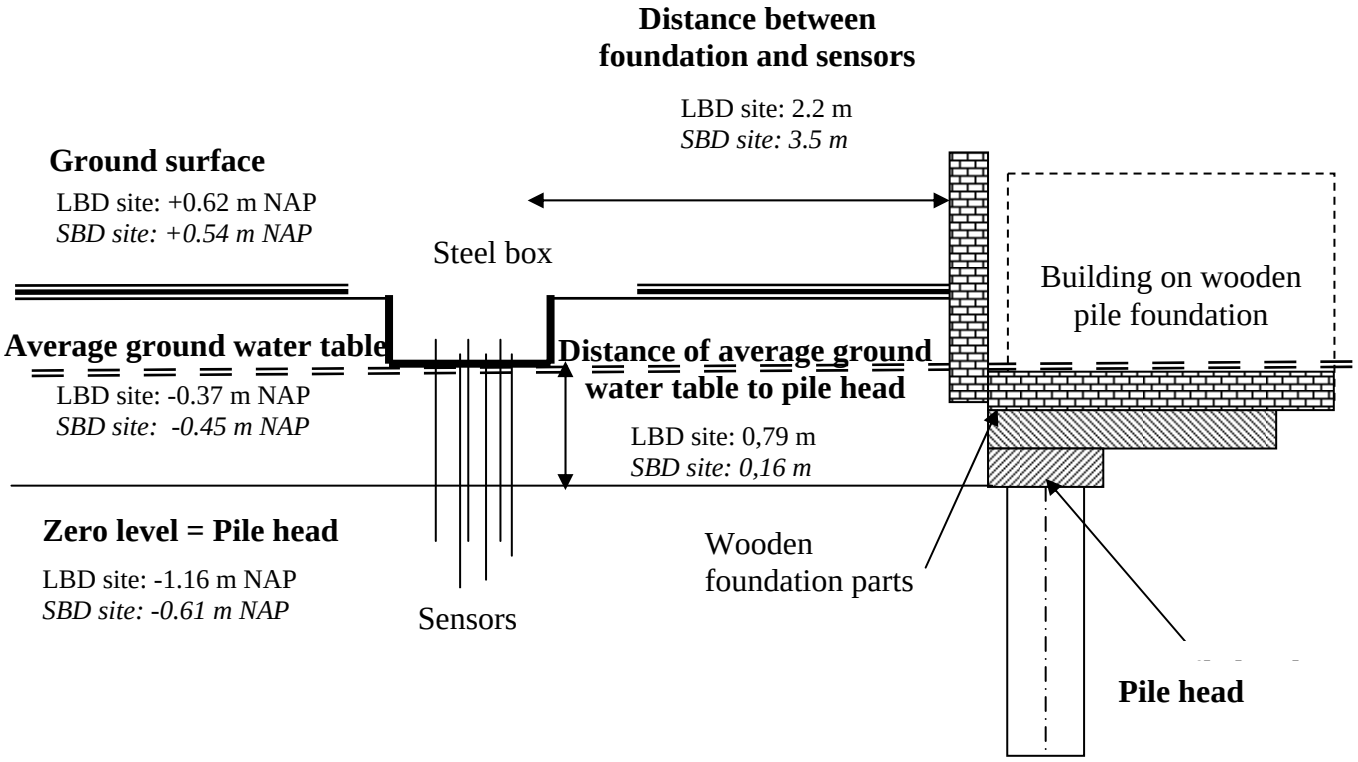

Fig. 3.4: Layout of field installations including levels (NAP = Normal Amsterdam Level) and depths of ground surface, water table, piles heads, and distances to the wooden pile constructions and buildings (for sensor installation depths see Tab. 3.2).

The oxygen sensors are installed in stainless steel tubes to facilitate installation and to protect the sensitive sensor tip from stones; for installation depths see Tab. 3.2. To minimise installation disturbances the tubes were flushed with nitrogen before inserting the oxygen sensors.

3.2.3. Sediment and Sediment water sampling

Before the sensors and the stand pipe were installed, sediment samples were taken with a hand auger, cooled and transported in the dark to the laboratory. Sediment water samples were obtained with suction lysimeter cups (P 80 ceramic material) which were installed three months prior to the first solution sampling allowing the sediment to equilibrate after positioning the sensor with a soil borer. Suction lysimeter cups where connected by PE-tubes to a glass bottle which was evacuated one day prior to single sampling regimes. Before first

samples were obtained, lysimeters were flushed and equilibrated to the chemical site conditions by discarding about half a litre of sucked sediment solution. Sampling of sediment solution was conducted at 4 single dates, covering all seasons of a year between late autumn 2003 and late summer 2004 (28.11.03, 26.02.2003, 28.05.2004 and 27.09.2004).

3.3. Microcosm experiment I

3.3.1. Microcosm set up

Fig. 3.5 shows the microcosm (MC) set up. In the gas inflow and outflow CO_2, N_2O and CH_4 were continuously monitored using a GC-ECD and FID (Shimadzu, Tokyo, Japan) described in Loftfield et al. (1997). Four treatments were used aiming to vary the oxygen availability in the sediment by altering the gas supply to the microcosm. The gas inflow was: 1. Air (A), 21% vol. O_2, 2. Air and oxygen (A+O_2), 50% vol. O_2, 3. Nitrogen (N_2), 0% vol. O_2. In the fourth variant water circulation by pumping the extracted water from the bottom to the top of the sediment column was combined with air inflow (A+C).

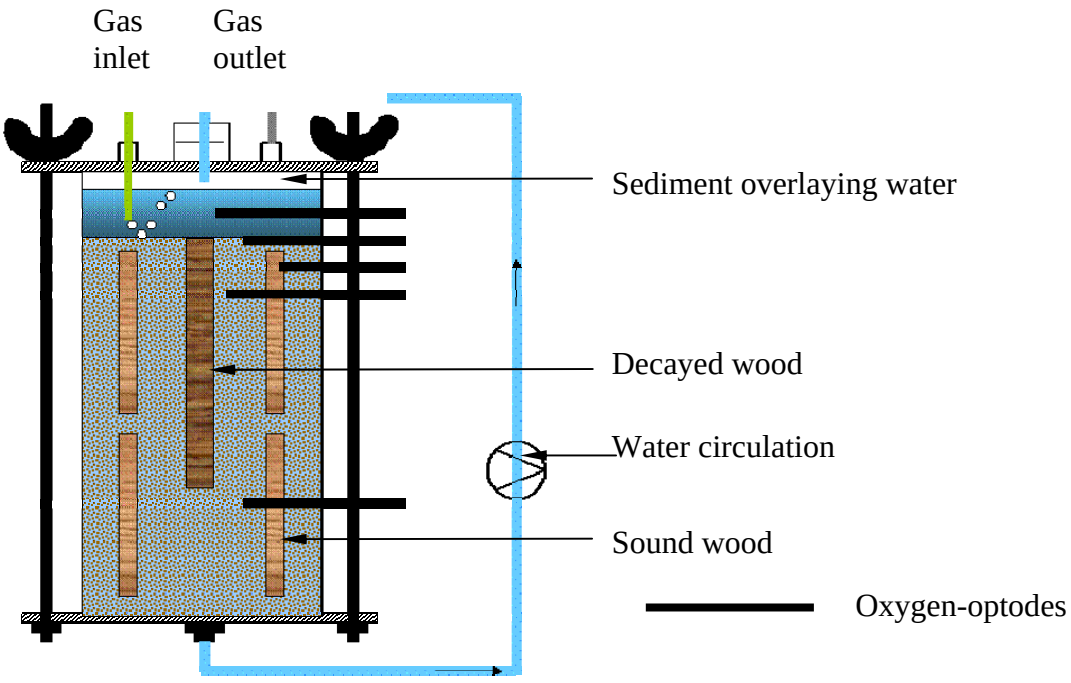

Fig. 3.5: Microcosm scheme with gas supply, water circulation and oxygen-optodes.

Twenty sound pine sapwood sticks with dimensions of 5 x 10 x 100 mm (decay control) together with a moderately decayed pine sapwood stick as an additional source of bacteria were incorporated into natural i.e non-sterilised sediment derived from a heavily decayed pine pile foundation site in the south of Amsterdam. The moderately decayed pine wood originated from a foundation pile extracted from a house in Koog an de Zaan/ in The Netherlands. Controls for the sediment gas production were sediment columns without wood which were subject to the same gas supply treatment regimes. For the experimental treatment regimes four replicate microcosms were used. For the control with air treatment three replicates were used and for the other treatment controls only one MC was used. For basic chemical composition of sediment, water, sound and infected wood used in the experiment see Tab. 3.3.

The microcosms (MCs) were incubated in the dark at 20°C. The MCs were harvested consecutively after 120, 150, 195 and 350 days. After 120 and 150 days one MC per experimental treatment was opend and after 195 and 350 days two MC per tretment were opend, with the exception that for the air treatment (A) after 195 days only one MC was harvested. Harvesting took place in a glove box under a nitrogen atmosphere by extracting the former sound wood together with sediment and water samples.

Tab. 3.3: Chemical sediment characteristics and mean (n=48, ±SD) mass of sediment, infected and sound wood and volume of water incorporated in the microcosms (MCs).

	Sediment [mg g^{-1}]	$Wood_{infected}$	$Wood_{sound}$		Water [mg L^{-1}]
C_{org}	3.5	452	485	DOC	56.5
N_t	0.1	1.3	1.2	N_t	3.6
P_t	0.2	0.053	0.033	PO_4^{3-}	0.5
S_t	0.2	1.8	0.034	SO_4^{2-}	21.6
pH	8.3			pH	8.1
				Conduct.	658 [µS/cm]
	[g]				[L]
Dry mass	6990 ± 220	5.3 ± 0.3	237.7 ± 4	Volume	1.75 ± 0.09

In the first stage of the microcosm experiment sound pine sapwood samples with dimensions of 5 x 10 x 100 mm were water saturated and arranged in two layers (top and bottom) in the MC sediment. In each layer 20 samples were located in two circles as shown in Fig. 3.6.

Fig. 3.6: Arrangement of wood samples in the upper layer of a microcosm (a plastic stick was used in the middle of the MC as placeholder for the later added decayed wood)

3.3.2. Oxygen measurements

During the experiment the oxygen concentration in selected microcosms was measured with oxygen optodes (PreSense, Regensburg, Germany). The Optode principle is the dynamic quenching of luminescence measuring the luminescence lifetime of a luminophore immobilised in a sensor foil (Klimant et al. 1995). A circle of 3 mm diameter of the sensor foil (PSt3-PSUP-YOP) is attached with silicone to the outer site of a closed plane end of a glass rod. The prepared glass rod is inserted into the microcosms with silicone rubber tubing as a gasket. For carrying out measurements a polymer optical fibre connected to the measurement device (Fibox 2-AOT) is held on the outside of the glass rod against the sensor foil which is inside the microcosm. Before installation, the optodes were calibrated using water saturated air as 100% and water saturated N_2 as 0%. Long-term stability was checked by re-calibrating a spare optode kept in the same room at similar conditions.

3.4. Microcosm experiment II

3.4.1. Microcosm set up

Glass jars of 500 mL volume with modified twist off lids were used in the second microcosm experiment (Fig. 3.7). The microcosms were connected to an automated GC-ECD and FID (Shimadzu, Tokyo, Japan) described in Loftfield et al. (1997) for continuous CO_2, N_2O and CH_4 measurements and aerated at a constant rate (approx. 20 mL min^{-1}) for 155 day (22 weeks) at 20°C in the dark.

Fig. 3.7: Glass jars with modified twist off lid with an in and out flow connection to the aeration and GC system and septum closed cylinder for water addition.

For each experimental treatment the MCs were filled with sediment, sound and infected wood and water to produce approx. 1 cm overlying water. Before the filling stage, the weight of each component was determined. The aeration was applied into the overlying water to circumvent the air-water-diffusion barrier. Each treatment had a control without wood addition. For the different treatments chemically modified sediment from Amsterdam was used together with the non-modified water from Amsterdam. Tab. 3.4 shows the different

chemical compositions of the sediment in the treatments. For each treatment 4 replicates were prepared.

Tab. 3.4: Different treatments, chemicals added and their anticipated concentrations in the sediment, dl = detection limit (0.1 mg g^{-1})

	Treatment	Addition	N	P	S
				[mg g^{-1}]	
S	Sediment (pure)	-	0.1	0.15	0.25
M	Mixture of 50 % sediment and 50 % silica sand	-		half	
SS	Silica sand	-		< dl	
S+A	Sediment + ammonium	NH_4Cl	1.0	-	-
S+N	Sediment + nitrate	KNO_3	1.0	-	-
S+P	Sediment + phosphorous	K_3PO_4	-	0.5	-
S+Su	Sediment + sulphate	K_2SO_4	-	-	0.5

To lower the sediment nutrient concentration it was “diluted” with silica sand (treatment M for mixture) or pure silica sand (SS) was used. The silica sand was prepared by successive washing with strong NaOH and HCl before rinsing it several times with distilled water. To study the effect of nutrient addition to the degree of bacterial wood decay, nitrate and ammonia were added to the sediment as nitrogen compounds as well as phosphorus. The last treatment had a sulphate addition to the sediment to assess the bacterial wood decay prevention potential of the chemical reactions in the sediment triggered by this addition.

Because of the reduced size of the microcosms compared to the MC experiment I, in this experiment smaller wood samples with dimensions of 10 x 5 x 30 mm were used. As in the first part of the experiment wood samples were water saturated and then added to the soil. In each MC, 10 sound pine sapwood samples were arranged in one circular layer and to ensure rapid infection, a piece of pine sapwood moderately decayed by bacteria was placed in the middle of each MC.

At the end of the incubation time the MCs were opened, the wood samples removed and the pH in the overlying water measured.

3.4.2. Sediment and water sampling (experiment I and II)

The microcosms were filled with sediment and water from a heavily decayed pine pile foundation site in the south of Amsterdam (for basic sediment and water characteristics see Tab. 3.3). The ground water saturated sediment was taken as bulk samples in buckets at the height of the head of the foundation piles. The water was ground water extracted with a pump and transported together with the sediment in lidded buckets. Before the start of the experiment the sediment and water samples were stored in buckets at 4° C. The microcosms were packed by hand and shaken when a certain height was reached to ensure even sediment compaction. The wood samples where inserted during filling of the acrylic glass cylinders with sediment. The sediment was overlaid with water to maximise gassing efficiency by applying the aeration into the overlying water, hence, circumventing the gas to liquid diffusion barrier.

3.5. Microcosm experiment III

3.5.1. Experimental design

The same microcosms as in the MC experiment I with the same treatments (A = Air, A+C = air and water circulation, N_2 = Nitrogen and A+O_2 = air and oxygen (50 vol%) were used. For the MC set up, sediment, water and wood sampling refer to the descriptions given in Experiment 1. The sediment was subject to additions of different chemicals which are listed in Tab. 3.5. From the remaining MCs of the experiment, four were equipped with oxygen optodes. In three MCs, five optodes and in one, ten optodes were installed. The incubation time was 4 weeks and two replicates per treatment (in total 8 replicates per addition treatment) were used.

Tab. 3.5: Different treatments of the MC experiment III and the chemicals added

Treatment	Addition per MC
Pure sediment as control	-
Sediment with glucose addition	20 mg glucose
Sediment with addition of glucose and sulphate	20 mg glucose + 20 mg K_2SO_4

Glucose and K_2SO_4 were dissolved in 200 mL water from Amsterdam (see MC experiment I for chemical water composition). The solution was inserted into the sediment with a syringe. First a 30 cm long stainless steel cannula, which was protected from clogging with sediment using an inserted thin metal rod, was pushed 25 cm deep into the MC sediment. Then, the metal rod was removed and a syringe was connected to the cannula. As the solution was introduced the cannula was slowly pulled out of the sediment to produce an even distribution of solution throughout the MC. For the control, the same procedure with water from Amsterdam was used. In some MCs it was necessary to remove some overlying water first before new solution could be added.

3.5.2. Kapok samples

In order to achieve bacterial wood decay over a short time period, kapok fibre was used as a surrogate for wood. Kapok is the seed enveloping fibre from the *Ceiba pentandra* tree and contains about 19 % lignin, i.e. it is typical lignocellulose comparable to wood (Fengel and Przyklenk 1986). Approximately 5 mg of kapok fibre was filled in bags made of polyester mesh sealed together by heating it. To facilitate recovery a string was attached to the bags. Four kapok bags were inserted into each MC. The MCs were opened and with the help of tweezers the kapok bags one after another, were pushed approximately 2 cm deep into the sediment. The kapok bags were positioned between the inner wood samples and the infected wood from the MC experiment I at the corners of a square. Kapok bags were recovered after four week by pulling at the string. In some cases the string failed and tweezers were used instead. However, in some cases not all four kapok bags could be extracted.

3.6. Sediment analysis

All sediment samples were analysed for pH, N_t (total nitrogen), C_t (total carbon), S_t (total sulphur), P_t (total phosphorus), and major cations (Ca^{2+}, Na^+, K^+, Mg^{2+}, Al^{3+}, Fe^{2+}, Mn^{2+}). Sediment samples were oven-dried at 40°C, sieved (2 mm) and their pH was measured with a digital pH-meter (WTW GmbH Weilheim, Germany) in water and 1 mol L^{-1} KCl (1:2.5). Sub-samples were ball-milled for N_t, C_t, S_t and P_t analysis. N_t and C_t was determined using an automated C and N analyzer (CHN-O-Rapide, VarioEL, Elementar, Hanau, Germany). Concentrations of S_t, P_t, Ca^{2+}, Na^+, K^+, Mg^{2+}, Al^{3+}, Fe^{2+} and Mn^{2+} were estimated after HNO_3 pressure digestion (65% HNO_3, see Heinrichs 1989) with the ICP-AES-technique, as described for water samples. Sediment texture was determined after sieving and using the pipette method.

3.7. Sediment water analysis

All water samples were measured for their pH, conductivity, dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and dissolved organic nitrogen (DON) contents, NH_4^+, NO_3^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Al^{3+}, SO_4^{2-}, PO_4^{3-}, and Cl^-. DOC measurements were made by dry combustion at 680 °C using a TOC-5050 Shimadzu organic C analyzer (Shimadzu Europa, Duisburg, Germany).

NH_4^+ and NO_3^- concentrations were determined using continuous flow injection colourimetry (Cenco/Skalar Instruments, Breda, The Netherlands). NH_4^+ concentrations were determined by the Berthelot reaction method (Skalar Method 155-000), whereas NO_3^- concentrations were estimated using the copper-cadmium reduction method (Skalar Method 461-00). Total dissolved organic nitrogen (TDN) estimates were made after converting NH_4^+ and DON to NO_3^-.by alkaline persulphate and UV digestion. Dissolved organic nitrogen was calculated using the following formula: [DON] = [TDN] – [NH_4^+-N] – [NO_3^--N].

The concentration of Cl^- ions was determined using a continuous flow system equipped with an Ag/AgCl ion selective electrode. The pH and the conductivity were measured with a digital pH/conductivity meter (WTW GmbH Weilheim, West-Germany). Concentrations of S_t, P_t,

Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Al^{3+}, Fe^{2+} and Mn^{2+} were estimated using an Inductive Coupled Plasma-Atomic Emission Instrument (ICP-AES, Spectro Analytical Instruments, Kleve, Germany), whereas those for SO_4^{2-}, PO_4^{3-} were derived from ICP S_t and P_t measurements respectively.

All water samples were checked for error by comparing the charge balance between positive and negative ions. Three Na^{+}, and two Cl^{-} analyses failed (from Borssele and the Venice sites, respectively) due to the high salinity of the water. For presentation purposes, they have been estimated from the charge imbalance.

3.8. Wood analysis

All wood samples were analysed on N_t (total nitrogen), C_t (total carbon), S_t (total Sulphur), P_t (total phosphorus), and major cations (Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Al^{3+}, Fe^{2+}, Mn^{2+}). Wood samples were oven-dried at 60°C and either ball-milled or consecutively milled by an ultra-centrifugal mill and a ball-mill. C and N contents were determined by an automated elemental analyzer (CHN-O-Rapide, VarioEL, Elementar, Hanau, Germany). S_t, P_t, Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Al^{3+}, Fe^{2+} and Mn^{2+} were analysed by ICP-AES (Spectro Analytical Instruments, Kleve, Germany) after pressure digestion in 65% concentrated HNO_3.

3.9. Data analysis

Data were analysed using Microsoft Office Excel 2003 or STATICTICA 6.1. The non-parametric Mann-Whitney U-test was performed for comparison of means of the total N content and the conductivity of the sediment water at the measurement sites in Amsterdam. For the cumulative CO_2-C production and the calculated wood derived CO_2-C production from the microcosm experiment I the Mann-Whitney U-test was as well applied. Linear regressions were carried out to analyse the relationship between the bacterial decay intensity and the incubation time, the CO_2-C production at the microcosm experiment I.

4 Sampling of the foundation pile surrounding

4.1 Results

Sediment colour varied with sediment origin: A black colour was noted for soils from sites that had a high organic content, such as those containing peat (present at the Borssele, Travenhorst and Rotterdam sites); soil from the two Venetian and Haarlem sites had a grey to blue-grey colour; whereas samples from sandy sites, such as Amsterdam and Zaadam, had a yellow sediment colour.

The environment surrounding the foundations varied according to the type of sediment present at the sites. Fig. 4.1 shows the variation of the sediment composition with depth at the different foundation sampling sites. Sediment from the archaeological sites Borssele (Roman) and Travenhorst (medieval) displayed high concentrations of total carbon (C_t) and total nitrogen (N_t), which corresponded to their high organic content due to the presence of peat.

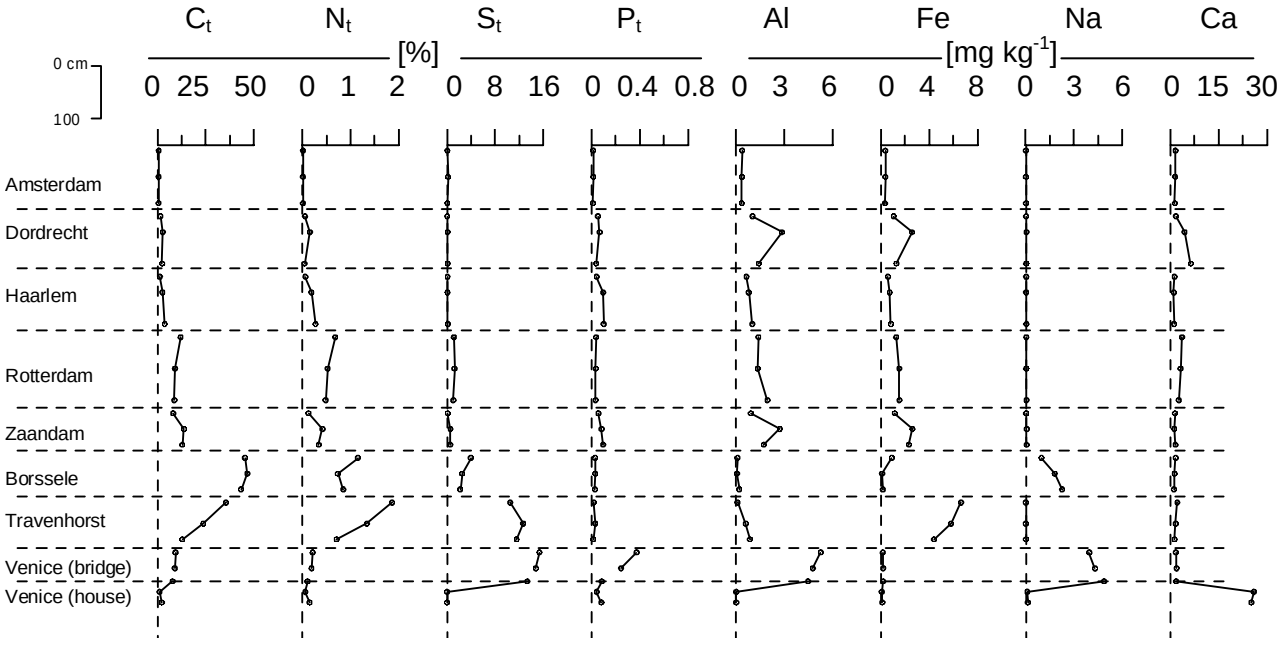

Fig. 4.1: Depth profiles of the chemical sediment composition at the sites investigated

Low C_t and N_t concentrations were recorded for soil samples from Amsterdam sites, whereas those from Rotterdam, Zaandam and the Venetian sites had medium concentrations. The total sulphur (S_t) concentration was greatest at the two sites with peaty sediment (Borssele and Travenhorst) together with the two sites in Venice which were of marine origin. Amsterdam,

Dordrecht and Harlem soil samples have low S_t concentrations, which is not surprising as they had low organic carbon contents and were not exposed to sea- or estuary water.

Total phosphate (P_t) concentrations were greatest for the two Venetian site samples, which reflected the fact that sewage is regularly discharged into the canals of Venice, increasing the eutrophic state of these two sites. Relatively high aluminium (Al) and iron (Fe) concentrations were recorded for samples from Dordrecht, Rotterdam and Zaandam. This could be taken to indicate the presence of clay minerals but pure clay was observed at the Rotterdam site only, whereas the Zaandam site was judged to comprise mainly sand overlaying peat. Interestingly, sulfur-rich sediments from the Venice bridge site and the first layer of the Venetian house site, exhibited relatively high aluminium concentrations but low iron concentrations, indicating that the sulfur was not present in the pyrite (FeS_2) form. The question of which sulfur-form pre-dominates in these samples remains unanswered. Sodium (Na) concentrations for most sampes are generally low with the exception of those from Borssele and Venice. Calcium (Ca) concentrations are only high in deeper layers at the Venice house site, and slightly elevated in Dordrecht and Rotterdam.

The pH of the sediment water (Fig. 4.2a, b) matches that of the carbonic acid/carbonate buffer range (pH 6.2 – 8.4); here, Ca^{2+} and HCO_3^- are the pre-dominant ions Fig 4.2b.

Differences exist between sites for sediment and soil concentrations of Na^+, K^+, Cl^-, and SO_4^{2-} ions, which can be attributed to whether or not the origin of the sample was marine or fresh water. Borssele and both of the Venice sites show relatively high concentrations of sodium (Na^+) and potassium (K^+), as well as of chloride (Cl^-) and sulphate (SO_4^{2-}) ions. The sulphate concentration at the Travenhorst site was also elevated. Magnesium (Mg^{2+}) is only elevated at the sites with marine influence.

The iron concentrations measured for water samples at different depths (Fig. 4.2a) were similar from all sites, ranging from 0 to 1 mgL^{-1}, with the exception of those from the deeper layer Haarlem samples, which reach levels 410 mgL^{-1}.

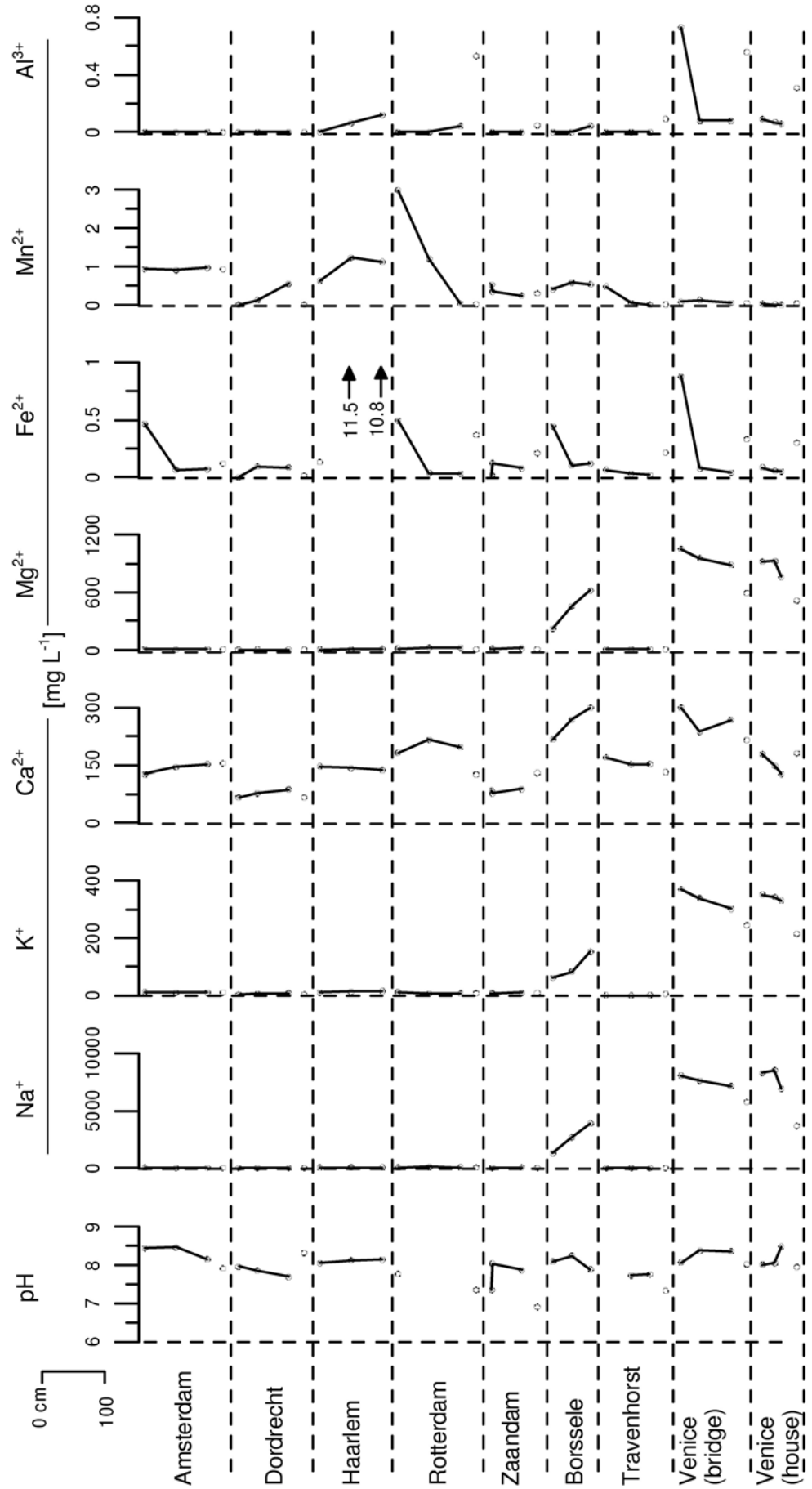

Fig. 4.2a: Depth profiles of the sediment pore water composition at the sites investigated. a.) for major cations and pH, full dots are samples taken with lysimeters, open dots are as surface water collected water samples.

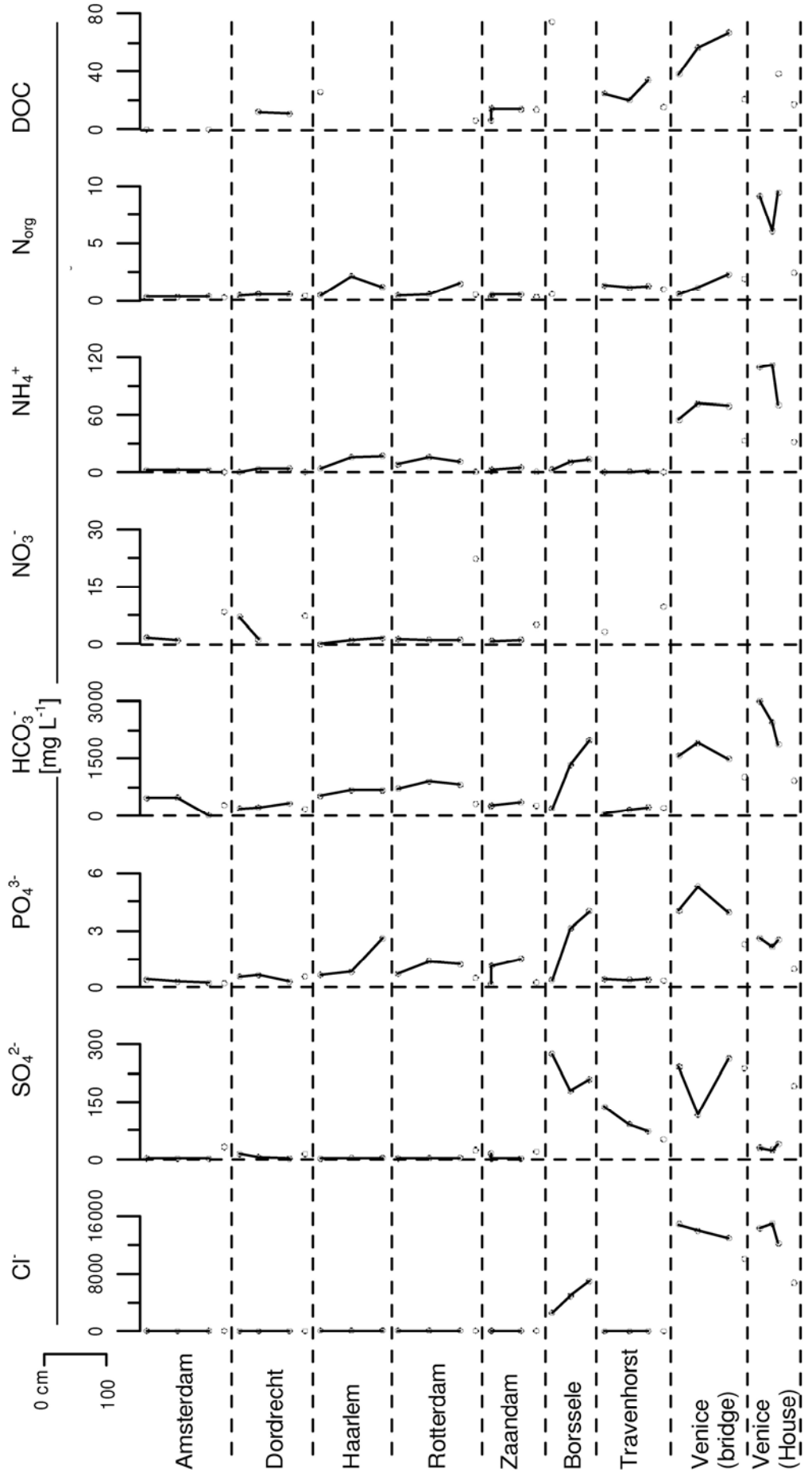

Fig. 4.2b: Depth profiles of the sediment pore water composition at the sites investigated. b.) for anions, N-compounds and DOC, full dots are samples taken with lysimeters, single dots are as surface water collected water samples.

When the magnesium concentration range was compared between the sites, differences were observed but on a much smaller scale than those recorded for the iron concentrations. The presence of soluble manganese and iron ions implies iron-reducing conditions. Higher aluminium (Al^{3+}) concentrations were found only at the Venice bridge site in samples above the pile head and at Rotterdam site in the water sample, which was collected as surface water.

Sites with high organic sediment content, such as Borssele and both Venice sites, showed high concentrations of phosphate in the samples taken. At the Venice bridge site this can be attributed to the release of sewage into the canal, which also accounts for a similar observation for the sample taken above pile head at the Venice house site.

The sewage release at these sites will also account for the high ammonium (NH_4^+) concentrations, as well as the lack of nitrates and the low redox potential values, recorded from all venetian samples. The lower ammoinium concentrations and increased nitrate concentrations observed for water samples (collected as surface water) at other sites suggest that the conditions there were less reducing ones than those for the Venetian sites. In the case of the Dordrecht site, this decrease in reducing conditions might be related to the presence of oxygen in the sample above pile head. The organic nitrogen (N_{org}) and the dissolved organic carbon (DOC) concentrations mirror the organic origin of the Travenhorst site and the high organic content at both Venice sites.

The change in redox potential with depth at the different sites is shown in Fig. 4.3. Samples from Amsterdam and the upper layer of Travenhorst and Borssele sites were the only ones where positive redox potentials were measured. The high values in the upper parts of the profiles from Borssele and Travenhorst are probably due to intrusion of oxygen during excavation. At the other sites, the range of redox values varied from 0 to -350 mV, with the lowest values recorded for the deeper samples indicating that the environment here was a reducing one.

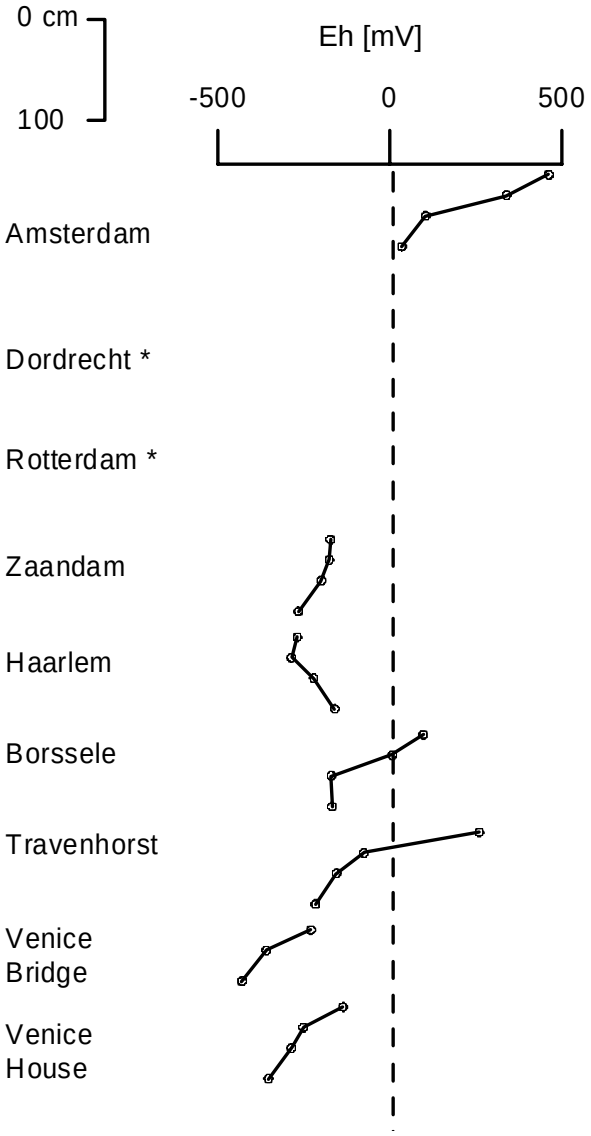

Fig. 4.3: Depth profiles of redox potential measurements.

* For this sites no measurements are available

4.2 Discussion

The sediment around wooden pile foundations was physico-chemically characterised to identify bacterial wood-decay favouring conditions. Sediment sodium concentrations were generally very low with the exception of samples from Borssele and Venice. At Venice, sodium from seawater seems to have adsorbed onto the organic matter. This phenomenon was not observed in deeper layers from the Venice (house) site, where no brackish water had reached the foundations at this depth. It is interesting to note that the steep decline in Na_t concentration with depth at the Venice house site coincides with an increase in the calcium (Ca_t) concentration. The high calcium concentration could indicate the presence of calcite and/or shell material formed at the bottom of the lagoon.

The marine or freshwater origin of samples from the sites can be best demonstrated by using a Piper plot (Piper 1944; see also Huisman et al. 2007a). A Piper plot (Fig. 4.4) was constructed that consisted of a two triangular diagrams with cations (Ca^{2+}, Na^{+}+K^{+}, Mg^{+}) concentrations plotted at each corner in the left-hand side triangle and anions (HCO_3^{-}, Cl^{-}, SO_4^{2-}) concentrations in the right-handed side triangle. In the central diamond shaped figure, anions and cations are combined by parallel projection to the outer sides of the figure.

Four different water types characterise each corner of the central, diamond shaped figure. Fresh water – usually dominated by Ca^{2+} and HCO_3^{-} – is described in the lower left portion of both the triangles and left sector of the diamond. Saline water – dominated by Na^{+} and Cl^{-}- is recorded in the lower right side of the triangles and the right part of the diamond. If sediment containing saline water is exposed to fresh water conditions (or visa versa) the anions are quickly exchanged from the sediment matrix while the cations, which bind more strongly to clay minerals, organic matter and oxides/hydroxides, take longer to move. This behaviour results in a non-linear transition of ions from freshwater to saltwater conditions in the diamond shaped diagram; where freshwater conditions will be described by the accumulation of points towards the lower corner or towards the upper corner if the salt water influence increases (Appelo and Postma 2005).

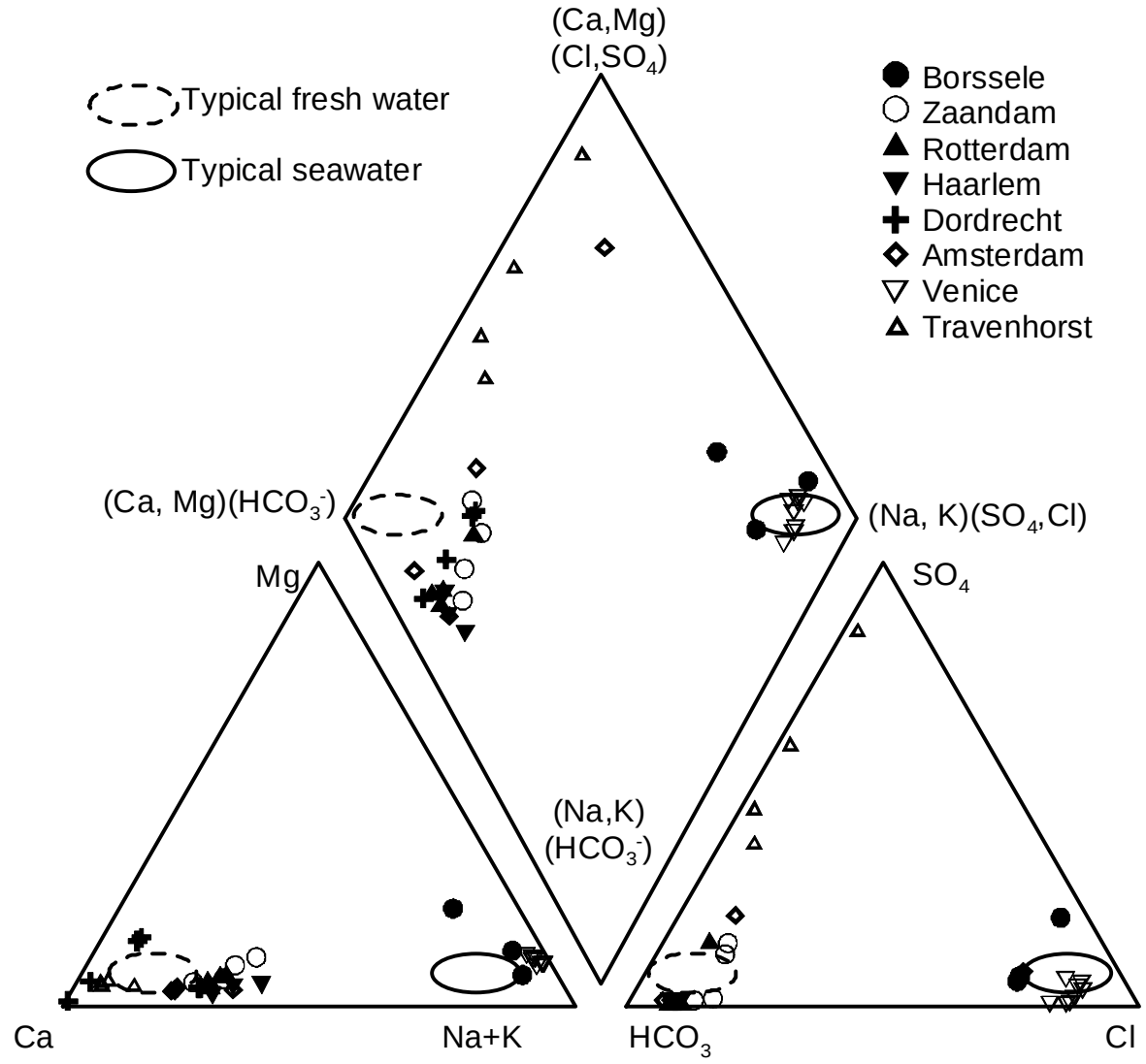

Fig. 4.4: Piper plot, characterizing the water composition. Normal fields for saline and fresh water are indicated.

In the Piper plot (Fig. 4.4) the different saltwater status of the sites can be clearly seen. Borssele and Venice are the only sites with a strong saline influence that is indisputable. At the Travenhorst site a transition from saline to fresh water composition has occurred, whereas many of the fresh water sites like Zaandam, Amsterdam, and Dordrecht show a slight tendency to more saline conditions. This latter description corresponds well with problems that have occurred in The Netherlands as a result of salt-water intrusion due to extensive ground water pumping. The transition from seawater to freshwater at the Travenhorst site,

predicted from the Piper plot, indicates that the peat at that location may have formed under marine or brackish conditions. If this maritime-formed peat was then incorporated into land near the river Trave, the exposure to a freshwater environment can be explained. This explanation excludes the possiblility that the sediment origin at the Travenhorst site was a result of sea spray deposition. If this were so, the high total sulphur concentration recorded for the Travenhorst sediment would be counter-balanced by a high chloride concentration, and this is not observed. Presumably, under the scenario where peat was formed in a maritime environment, the pyrite present in the sediment is now under a freshwater influence, which will favour pyrite-oxidation reactions leading to the release of sulphate.

The measured positive redox potential in Amsterdam is probably a sampling artefact. The pile heads at the sites are surrounded by sand. In order to reach the foundation piles it was necessary to vigorously extract ground water by pumping. This could have facilitated air oxygen intrusion. Therefore, it is not possible to judge the normally established redox potential. In Travenhorst, as well as at Borssele, the sites were peat covered but the upper sand or clay layer of the peat was removed before archaeological excavation. This is reflected in the higher redox potential in the upper layers of these sites. Further down in the peat profile reducing conditions are established which is also mirrored in the increasing ammonium concentration at the Borssele site.

The Eh-pH diagram (Fig. 4.5) confirms the reducing conditions at the Venetian sites, Haarlem, Zaandam and the lower layers of the Borssele and Travenhorst sites. These sites reach values that are normal for iron and sulphate reducing environments in which free oxygen exists in small quantities (Appelo and Postma 2005). At the Amsterdam site and in the upper layers of the Borssele and Travenhorst sites the conditions during sampling were of a less reducing nature than elsewhere. (see also Fig. 4.3).

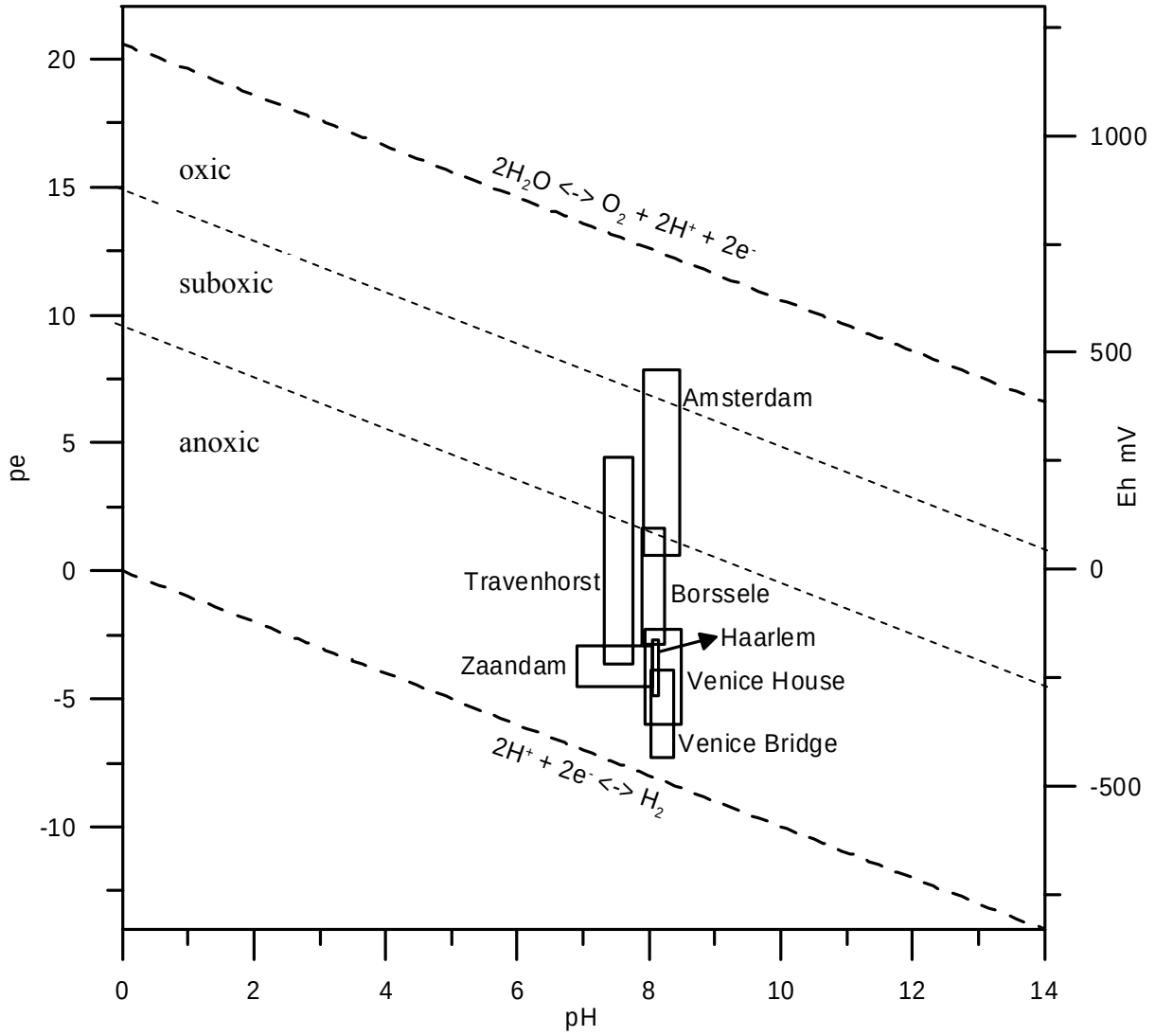

Fig. 4.5: Eh–pH diagram, showing the range of Eh and pH at each site. Since the depths of measurement for Eh and pH were different at most sites, it was not possible to plot sample points in this diagram. Therefore, the range of variation in Eh and pH between the sample points is shown. All sites except Amsterdam and Travenhorst reach the field of iron- and sulphur-reducing conditions. The Amsterdam site and upper samples of Travenhorst reach into the oxic zone with Eh over 200 mV (see also Fig. 4.3).

Bacterial wood decay was detected at all sites but with different orders of magnitude (Tab. 4.1). The only site with remarkably little bacterial wood decay was Rotterdam. Here the spruce piles were degraded slightly in the outer, sections only.

Tab. 4.1: Wood species, classification of bacterial decay patterns and occurrence of wood colonising fungi and softrot decay in wood extracted from the different sampling sites after Klaassen et al. 2005

Site	Wood species sampled	Bacterial wood degradation intensity	Fungal hyphae	Softrot *
Amsterdam	Pine / Spruce	Sapwood (55 mm) severely, gradient half moderate to weak	Only outside	-
Dordrecht	Spruce/ Pine	In 20 – 30 mm gradient from severe to weak, Pine sapwood only (80 mm) gradient	Only outside	1/12
Haarlem	Pine	Sapwood only severe	In decayed area	1/7
Rotterdam	Spruce / Fir	Small layer (10 mm) moderate decay	-	1/3
Zaandam	Pine	Sapwood only, severe gradient to weak, depth gradient in pile top	Whole area	1/3
Borssele	Oak	Severe sapwood, heartwood 1 pile severely other pile weak	Only outside	-
Travenhorst	Oak/ Spruce	Whole pile severe (outside) to moderate (inside)	Only outside	-
Venice, Bridge	Fir / Oak / Larch	Severe outside, gradient moderate to weak inside	Only outside	-
Venice, House	Oak/ Pine	Severe outside, gradient moderate to weak inside	-	-

* Number of piles affected by soft rot/ number of piles sampled

Investigation of extracted piles resulted in comparable bacterial wood decay along the entire pile length (Klaassen 2007a). Therefore, it is possible to derive conclusions from the investigation of the sediment conditions around the pile head only; not along the whole pile length. Furthermore, the investigation of bacterial wood decay suggest that it is present in nearly all condition but that the velocity the decay proceeds is dependent from the environmental conditions (Huismann et al. 2007a; Klaassen 2007a).

It was not possible to compare all of the sampling sites for the effects of nitrogen and phosphate concentrations on the extent of decay caused by erosion bacteria. Any relationship between these levels is likely to be masked because of the complex interactions that exists between bacterial populations, the age of the pile and its composition, and the sediment type within which the foundation was buried. Nevertheless, a subgroup of sample sites with sandy sediment types (Amsterdam, Dordrecht and Zaadam) could be compared with two similar sites in Amsterdam where nitrogen and phosphate concentration measurements and wood

decay indicies have been recorded over a long time period (see Kretschmar et al. 2007b). When data from these sandy soil sites were grouped together (Tab. 4.2) it was evident that nitrogen concentrations in sediment water decreases with increasing bacterial decay intensity. This pattern was not observed for phosphate concentrations in the sediment water.

Tab. 4.2: Sandy sediment sites: sediment water phosphate, dissolved organic carbon (DOC), total N, bacterial decay intensity and pilodyn penetration depth

Site	PO_4^{3-}-P	DOC	N_t	Decay	Pilodyn penetration depth *
	[mg L^{-1}]				[mm]
Little Bacterial decay	0.38	13.6	6.0	++	15
Dordrecht	0.53	11.4	2.8	+	9
Zaandam	0.78	12.0	2.4	++	11
Amsterdam	0.29	-	1.7	++++	40
Severe Bacterial Decay	0.38	7.7	1.7	++++	>50

* Pilodyn penetration depth is a measurement for wood decay (higher values indicate higher decay). In this case it can be taken as a surrogate for bacterial wood decay as the microscopic investigation showed mostly bacterial decay pattern in the wood.

The results of this survey are comparable with long-term measurements in Amsterdam at two differently bacterial decayed wooden foundations (Chapter 5): This observation suggests that, at least for sandy sediments, bacterial decay may be reduced when the level of nitrogen is elevated. The mechanisms for this reduction in the bacterial decay process are unknown. Furthermore, different sediment types may have different decay-favouring mechanisms. In clay soils, higher bacterial decay was associated with higher nitrogen and phosphate concentrations in sediment and sedimental water (Boutelje and Göransson 1975).

5 Investigating chemical sediment conditions at bacterial decayed wooden pile foundation sites in Amsterdam

5.1 Results

5.1.1 Sediment chemistry

Most of the elements analysed at both sites showed comparable levels (Tab. 5.1). Nevertheless, significantly ($p<0.05$) higher carbon, phosphor, sulphur and calcium concentration as well as slightly higher (sig. $p<0.1$) nitrogen concentrations were found at the LBD site.

Tab. 5.1: Sediment chemistry at pile head, total concentrations and C/N ratio mean values (n=4), pile head at light bacterial decay (LBD) site: -1.16 m NAP and severe bacterial decay (SBD) site: -0.61 m NAP (Normal Amsterdam Level)

	Depth	pH	C	N	P	S	C/N	Na	K	Ca	Mg	Mn	Fe	Al
	m NAP	H_2O	mg g^{-1}				-	mg g^{-1}						
LBD	-0.66	7.5	15.6	0.44	0.35	3.5	35.1	0.29	1.7	17.6	1.4	0.15	12.6	6.9
SBD	-0.66	7.6	12.1	0.36	0.25	0.49	33.7	0.25	1.6	9.0	1.1	0.10	9.8	6.2

5.1.2 Sediment water chemistry

Tab. 5.2 presents the seasonal variation of the sediment water chemistry for both sites. Values are given as means for depth (LBD site = -0.66, -1.21, -1.66 m NAP (Normal Amsterdam Level), SBD site =-0.31, -0.66, -0.76, -1.11 m NAP) per season and as mean over the total observation period and all sediment depths, further referred to as grand mean. With only two exceptions at the LBD site no ammonia was measured. At the LBD site the mean sodium concentration is significantly ($p<0.05$) higher (annual mean 77 mg L^{-1}) compared to the SBD site (annual mean 29 mg L^{-1}). This also holds true for the chloride concentration (annual mean 105 and 26 mg L^{-1} for the LBD and the SBD site respectively, sig. $p<0.05$). The differences in the total nitrogen concentration, reaching at the LBD site a grand mean of 6.0 mg N L^{-1} compared to 1.7 mg N L^{-1} at the SBD site are mostly caused by different nitrate concentrations. The grand mean organic nitrogen concentration is slightly lower at the SBD

site and seasonally less variable compared to the LBD site. The mean concentration of DOC (dissolved organic carbon) is at the 10% level significantly higher at the SBD site. The grand mean of conductivity shows comparable values of approximately 1000 µS cm^{-1} at both sites.

Tab. 5.2: Seasonal variation and annual mean of sediment water chemistry at the LBD and the SBD site. Repeated sampling and analysis was applied in Autumn (28.11.), Winter (26.02.), Spring (28.05.) and Summer (27.09.) 2003/2004. Mean values over the sediment profile, measurement depths correspond to n, LBD site n=3, SBD site n=4 and mean annual values per site (dl.=detection limit, n.a.=not analysed).

		Light bacterial decay (LBD)				**Annual**	**Severe bacterial decay (SBD)**				**Annual**	
		Autumn	Winter	Spring	Summer	**mean**	Autumn	Winter	Spring	Summer	**mean**	p<0.05
N_t	[mg L^{-1}]	4.7	5.7	6.7	7.0	**6.0**	1.0	2.0	1.6	2.1	**1.7**	sig.
NO_3^--N	[mg L^{-1}]	3.0	5.3	2.7	6.2	**4.3**	0.8	1.8	1.4	1.8	**1.4**	-
NH_4^+-N	[mg L^{-1}]	1.3	0.6	<dl.	<dl.	**0.47**	<dl.	<dl.	<dl.	<dl.	**<dl.**	-
N_{org}	[mg L^{-1}]	0.48	0.36	0.49	0.20	**0.38**	0.25	0.20	0.21	0.27	**0.23**	-
DOC	[mg L^{-1}]	13.1	19.6	10.2	11.5	**13.6**	7.0	23.8	<dl.	<dl.	**7.7**	sig.
Conduc-tivity	[µS cm^{-1}]	988	956	1164	1090	**1050**	628	770	1438	1099	**984**	-
SO_4^{2-}-S	[mg L^{-1}]	1.6	2.1	1.7	1.7	**1.8**	5.1	12.2	33.3	24.7	**18.8**	sig.
PO_4^{3-}-P	[mg L^{-1}]	0.34	0.41	0.36	0.39	**0.38**	0.38	0.41	0.38	0.35	**0.38**	-
Cl^-	[mg L^{-1}]	108	102	n.a.	n.a.	**105**	25	26	n.a.	n.a.	**26**	sig.
Na^+	[mg L^{-1}]	72	67	82	86	**77**	17	18	34	50	**29**	sig.
K^+	[mg L^{-1}]	16.3	15.1	19.3	21.2	**18.0**	10.8	11.0	18.0	20.6	**15.1**	-
Ca^{2+}	[mg L^{-1}]	108	111	130	129	**120**	99	149	200	256	**176**	sig.
Mg^{2+}	[mg L^{-1}]	10.5	10.4	11.7	11.5	**11.0**	6.0	8.4	11.8	15.2	**10.3**	-
Fe^{2+}	[mg L^{-1}]	0.33	0.14	0.33	0.52	**0.33**	1.1	0.4	0.02	0.03	**0.38**	-
Mn^{2+}	[mg L^{-1}]	0.36	0.46	0.36	0.51	**0.42**	0.31	0.42	0.43	0.75	**0.48**	-
pH		8.2	7.7	7.8	8.0	**7.9**	8.1	7.9	7.7	7.9	**7.9**	-

Separated for single sediment depth the annual total nitrogen concentration (Fig. 5.1) does not show any strong depth gradient or seasonal variations between sites. The total nitrogen concentration is dominated by the nitrate N-fraction (72-82% of total N) and increasing nitrate concentrations were detected with increasing sediment depths at the LBD site (Fig. 5.2). The repeated measurements indicate slightly (SBD site) to significantly ($p<0.05$, LBD site) increased nitrate concentrations at both sites in winter and summer samples. Separated for single seasons, Fig. 5.3 indicates that there is no obvious time pattern for the nitrate concentrations measured at the LBD site. This is also the case at the SBD site (not shown).

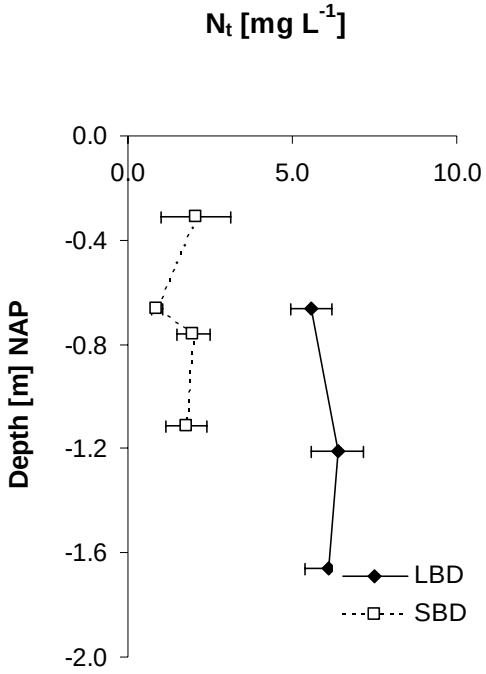

Fig. 5.1: Mean depth gradient of total nitrogen in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

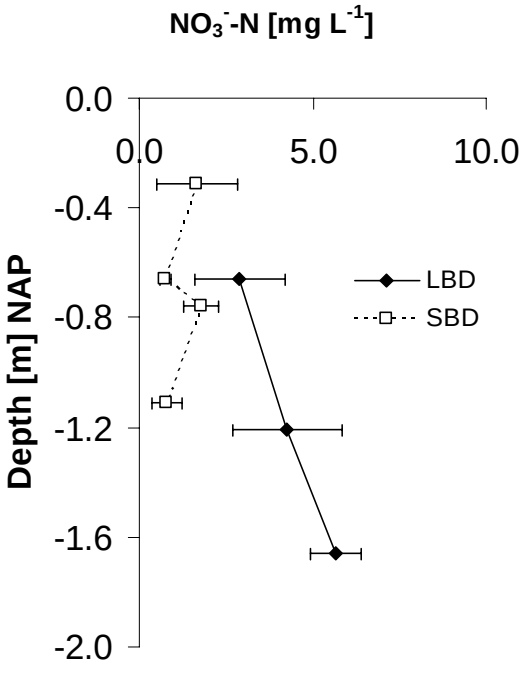

Fig. 5.2: Mean depth gradient of nitrate concentration in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

The grand mean organic nitrogen concentration (Fig. 5.4) is slightly lower at the SBD site and seasonally less variable compared to the LBD site. There is no seasonality in the organic nitrogen concentration at both sites (not shown).

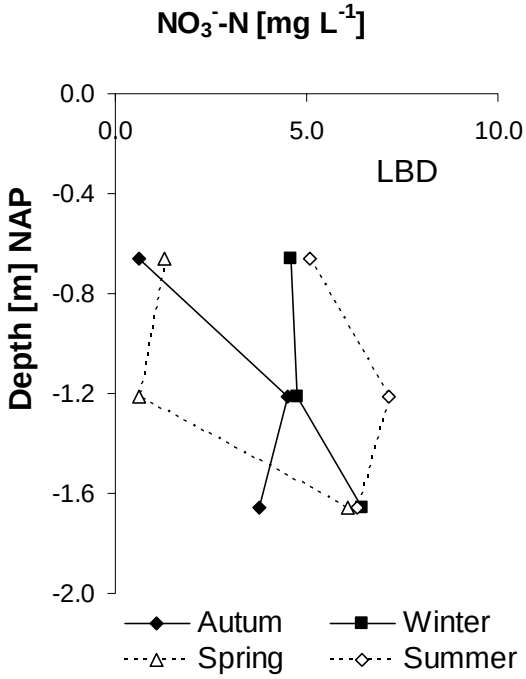

Fig. 5.3: Vertical gradients of single (n=1) nitrate measurements in sediment waters at the LBD site, quarterly separated samplings: Autumn 03 to Summer 04.

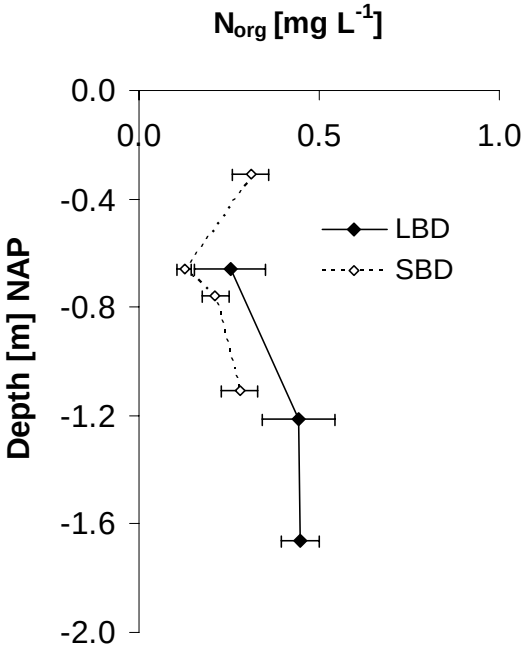

Fig. 5.4: Mean depth gradient of organic nitrogen concentration in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

The mean concentration of DOC (dissolved organic carbon, Fig. 5.5) is at the 10% level significantly higher at the SBD site but does not indicate any strong changes within the observed depths gradients at both sites. For the SBD site however, significant ($p<0.05$) seasonal variations on the one hand between the autumn and winter period and on the other hand the spring and summer period were analysed.

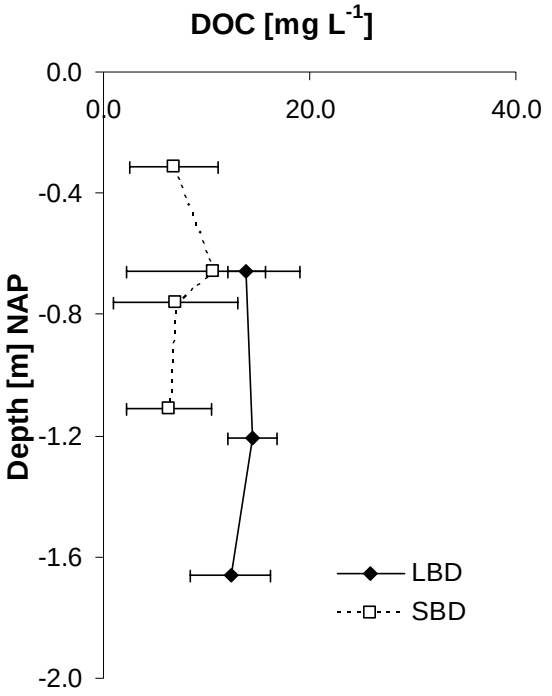

Fig. 5.5: Mean depth gradient of dissolved organic carbon (DOC) in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

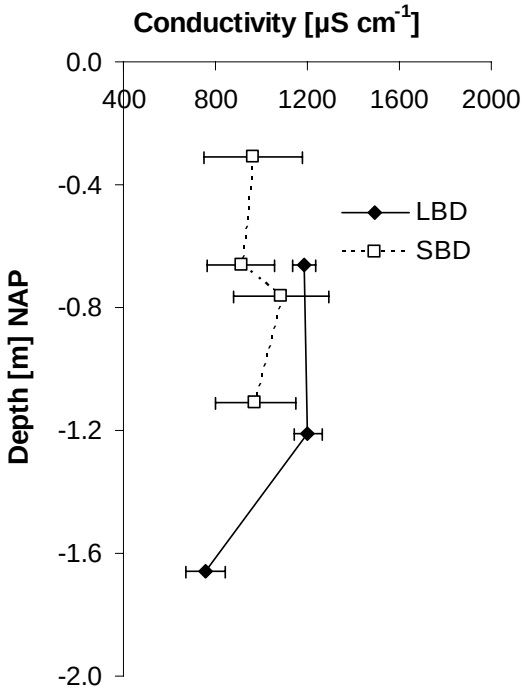

Fig. 5.6: Mean depth gradient of conductivity in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

The grand mean of conductivity (Fig. 5.6) shows comparable values of approximately 1000 µS cm^{-1} at both sites. However, decreasing values were detected in the lowest depth at the LBD site. Furthermore the SBD site displays a higher SE as compared to the LBD site. Fig. 5.7 exhibits the conductivity within the profile, separated for the single samplings at the SBD site. A significant ($p<0.05$) seasonal variation of conductivity with higher values in spring and summer compared to autumn and winter samples was analysed. At the LBD site (not show) the conductivity showed a homogeneous course over the same periods.

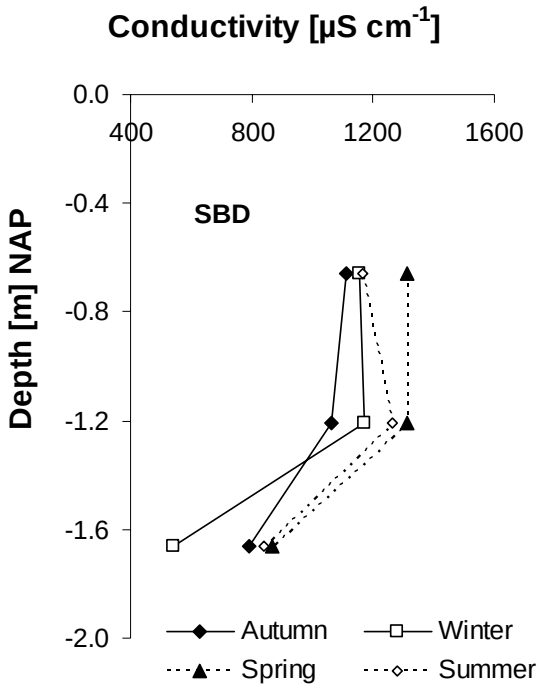

Fig. 5.7: Vertical gradients of single (n=1) conductivity measurements in sediment waters at the SBD site, quarterly separated samplings: Autumn 03 to Summer 04.

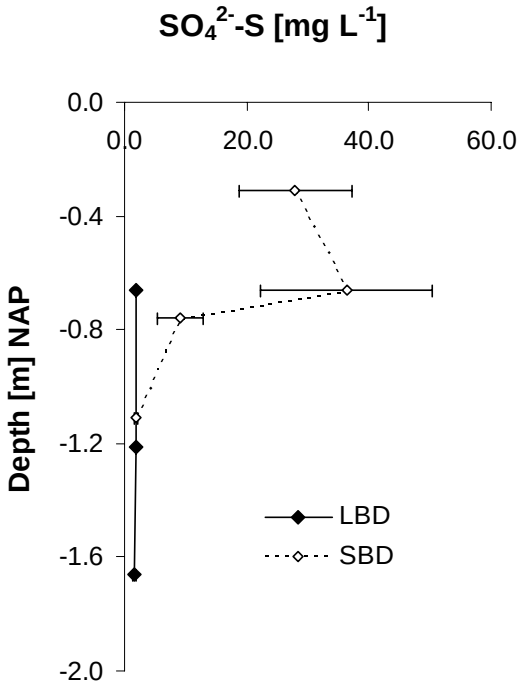

Fig. 5.8: Mean depth gradient of sulphate in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

Different ions were identified governing the conductivity at the two sites. At the SBD site the sulphate concentration (Fig. 5.8) was significantly higher compared with the LBD site. However, sulphate concentrations were decreasing to the values of the LBD sites in deeper sediment layers. At the SBD site calcium (Fig. 5.9) and sulphate were the main ions present. These two ions show separately the same seasonality as the conductivity values. Nevertheless, there is no obvious time pattern in the seasonal variations of the sulphate concentration at the SBD site.

At the LBD site the mean sodium concentration (Fig. 5.10) is significantly ($p<0.05$) higher (annual mean 77 mg L^{-1}) compared to the SBD site (annual mean 29 mg L^{-1}). This holds also

true for the chloride concentration (annual mean 105 and 26 mg L^{-1} for the LBD and the SBD site respectively, sig. $p<0.05$, not shown). No seasonality was detected in the sodium and chloride concentrations (not shown) at the LBD site which is also the case for the conductivity at the LBD site.

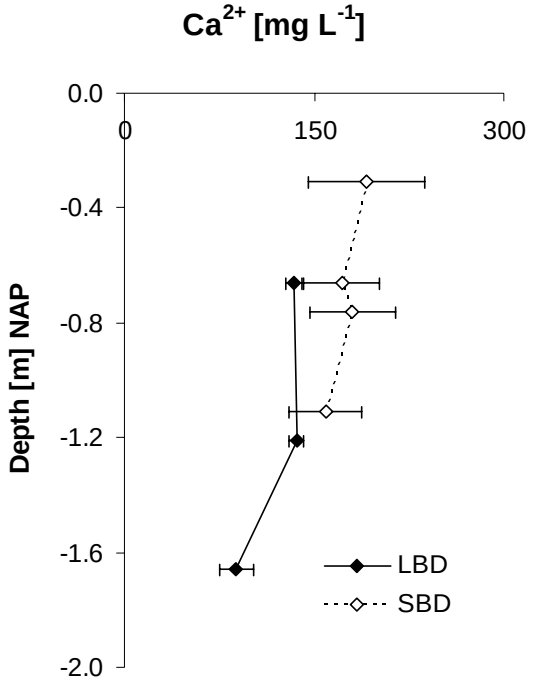

Fig. 5.9: Mean depth gradient of calcium in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

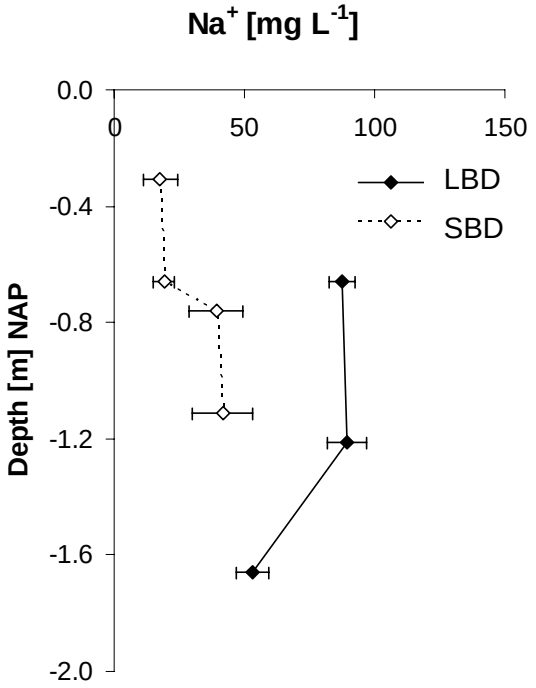

Fig. 5.10: Mean depth gradient of sodium in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

The mean phosphate concentration (Fig. 5.11) at the two sites is comparable and does not indicate strong seasonal variation. The mean iron concentration (Fig. 5.12) displays a high seasonal variability at the SBD site which cannot be attributed to a distinct time pattern.

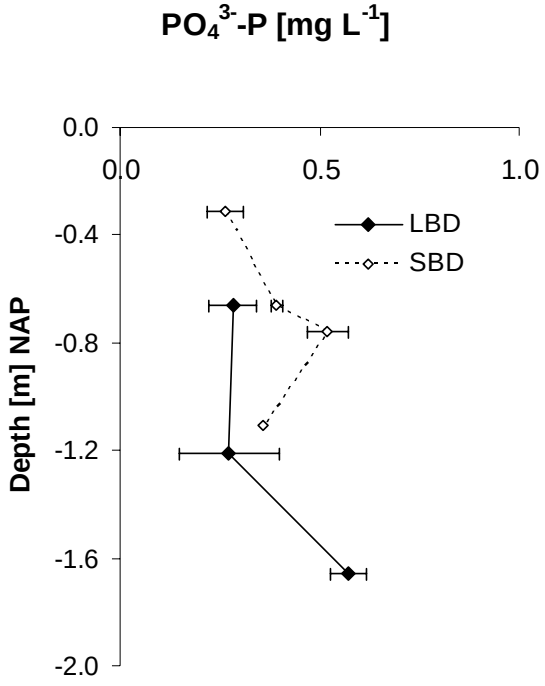

Fig. 5.11: Mean depth gradient of phosphate in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

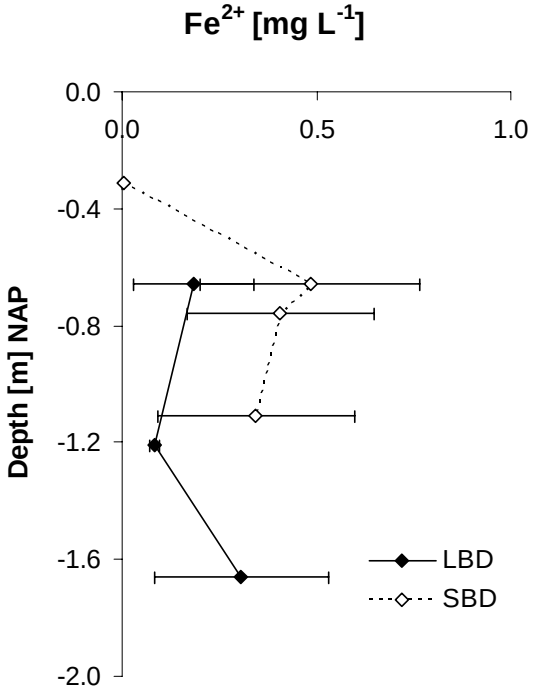

Fig. 5.12: Mean depth gradient of iron in sediment waters at both sites. The standard error per depth indicates the seasonal variation within the observation period, quarterly single samplings: Autumn 03 to Summer 04.

5.1.3 Site Characteristics

5.1.3.1 Chemical site parameters

Oxygen concentrations (Fig. 5.13) range between 8 to 0 mg L^{-1}recorded at the LBD site from August 03 until September 04. After a three month settling period post first installation (June 03 – August 03) four out of nine sensors show oxygen concentrations ranging from 6 to 2 mg L^{-1}in various depth. Due to additional applied redox measurements these values were interpreted as O_2-contamination during first initial installation in June 03. Therefore, oxygen sensors were reinstalled in Dec 03. The reinstallation resulted in a marked decrease in the oxygen readings in all sensors. However, even after the reinstallation some of the oxygen

sensors still seemes to be O_2-contaminated and displayed enhanced O_2-values not in accordance with the redox measurements. Notably one of the triplicate sensors five centimetres below the pile head showed values up to 8 mg L^{-1} in March 04, whereas the two other sensors at this depth measured zero oxygen. Nevertheless, approaching the end of the observation period in August 04 all but one sensor 0.3 m below the pile head (-1.46 m NAP) indicated zero oxygen values.

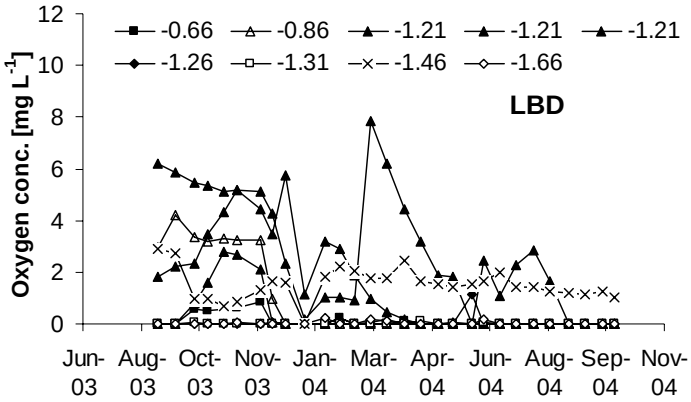

Fig. 5.13: Biweekly observed oxygen concentrations [mg L^{-1}] at the LBD site over the measurement period in different depths [m NAP] pile head at -1.16 m NAP.

At the SBD site (Fig. 5.14) very high oxygen concentration from 1.2 to 10.4 mg L^{-1} were measured from August 03 to December 03. The sensor reinstallation in December 03 resulted in a marked decrease down to zero values in all but two sensors. The sensor at the pile head (-0.61 m NAP) increased again after reaching zero following reinstallation and measured zero from Jun 04 on. One of the triplicate sensors at the pile head was not affected by reinstallation increasing slightly to 1 to 2 mg L^{-1} oxygen but showed zero values from June 04 on. The other seven sensors at various depths at the SBD site measured zero oxygen from March 03 onwards.

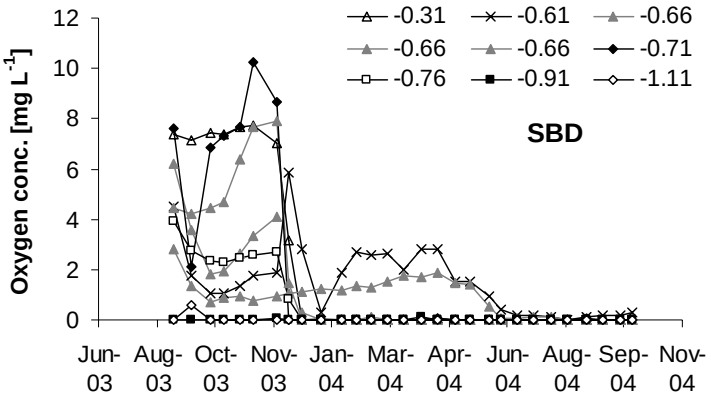

Fig. 5.14: Biweekly measured oxygen concentrations [mg L^{-1}] at the SBD site over the measurement period in different depth [m NAP] around the pile head at -0.61 m NAP.

All redox potential measurements (Fig. 5.15) did not show any strong special variability during the measurement time from September 03 – September 04. Values at both sites at the pile head and below are comparable and most of the time around -200 mV. At the start of the observation period in September and October 03 redox potential values above the pile head increased to zero and + 400 mV at the LBD and the SBD site respectively. A similar increase occurred in spring 04 only at the SBD site. However, towards the end of the observation period in August 04 values increased up to zero again at the LBD site above the pile head.

Light bacterial decay **Severe bacterial decay**

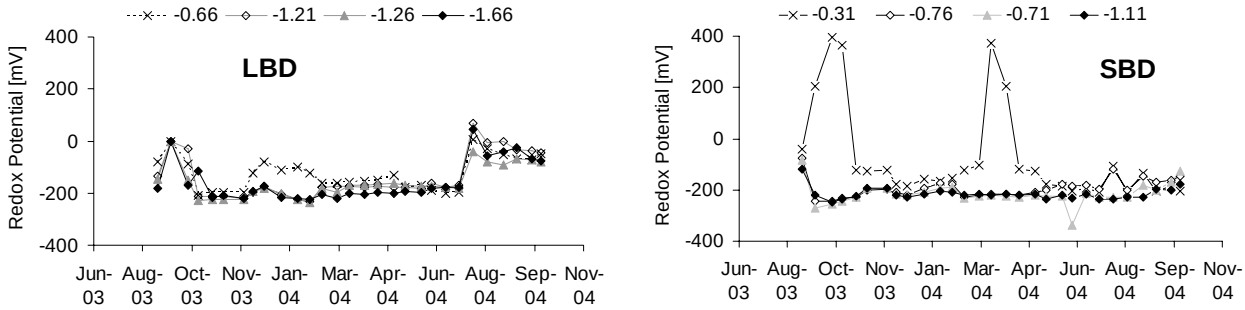

Fig. 5.15: Biweekly observed redox potential measurements [mV] at the LBD and the SBD site in four different depths [m NAP] during the measurement period (n=1).

5.1.3.2 Physical site parameters

The depth of the ground water table is shown in Fig. 5.16 and ranged from -0.30 to –0.72 m NAP at the SBD and -0.26 to -0.50 m NAP at the LBD site. The lowest ground water table during the observation period from September 03 to September 04 was recorded in September 03 for both sites. Over the entire measurement period the ground water level was lower at the SBD site compared to LBD site. Both sites displayed a clear seasonal pattern with low levels over spring and summer and high levels in autumn and winter. During the measurement period, the average ground water table of -0.37 and -0.45 m NAP for the LBD and SBD site, respectively, was at both sites 0.99 m below ground level.

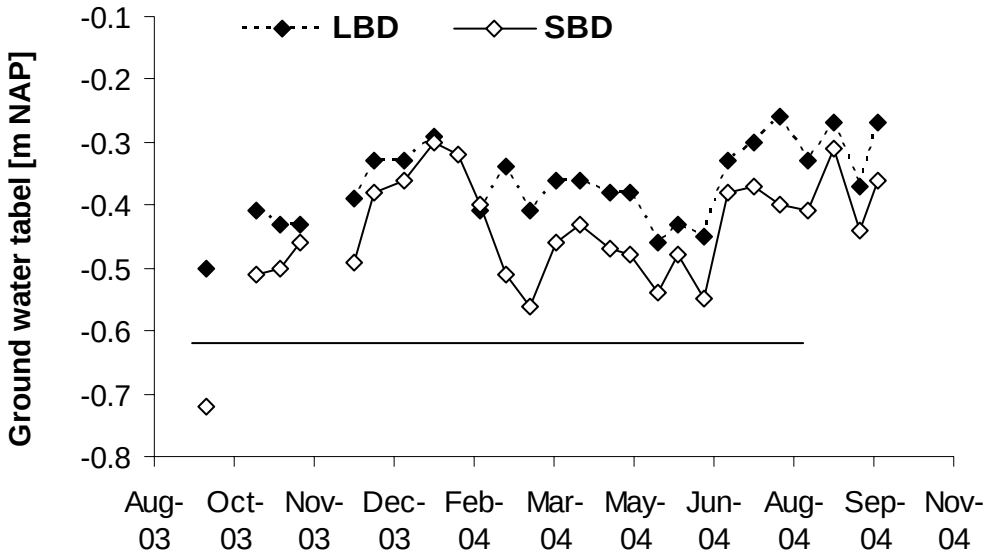

Fig. 5.16: Biweekly observed changes in ground water table between September 2003 until September 2004 at the LBD and the SBD site. The horizontal line indicates the level of the pile head at the SBD site (pile head LBD site at -1.16 m NAP, not shown).

The velocity of the horizontal water flow (Tab. 5.3) at the LBD site (mean 0.26 m day^{-1}) was slightly higher than at the SBD site (mean 0.21 m day^{-1}). At the LBD site the ground water flow was towards the northwest and at the SBD site towards the northeast. The direction of the water flow at the SBD site was as expected towards the Sarphati Park.

Tab. 5.3: Ground water flow direction and velocity at the pile head at both sites

	Light Bacterial Decay (LBD)		**Severe Bacterial Decay (SBD)**	
Date	Direction of water flow [^{0}] *	Velocity of water flow [m day^{-1}]	Direction of water flow [^{0}] *	Velocity of water flow [m day^{-1}]
11.06.04	324	0.27	47	0.19
11.08.04	324	0.24	23	0.22

*Direction: 0^0=North; 90^0=East; 180^0=South; 270^0=West

Mean sediment temperature (Fig. 5.17) was measured at the pile head for the SBD site and 0.3 m above the pile head at the LBD site between August 2003 and September 2004. The temperature ranged between 18.9 and 6.1° C and 20.6 and 8.1° C at the SBD and the LBD site respectively. As expected a clear seasonal pattern was observed with lowest values reached in March at the end of winter and highest temperature measured in September.

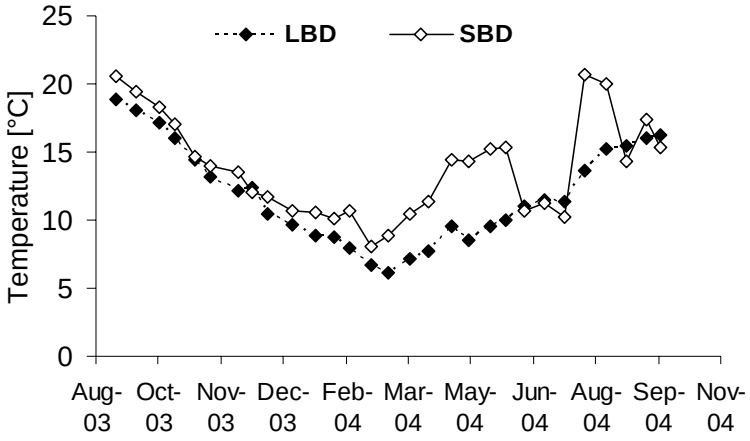

Fig. 5.17: Biweekly measured sediment temperature observed between September 2003 and September 2004, LBD site: 1.48 m below ground surface (-0.86 m NAP) and SBD site: 1.2 m below ground surface (-0.66 m NAP)

The average annual temperature, measured between September 2003 and August 2004, is 11.4° C at the LBD and 13.6° C at the SBD site. Compared to the long-term annual mean air temperature of 9.8° C measured in Schiphol close to Amsterdam (mean 1971-2000, Royal Netherlands Meteorological Institute) it is slightly higher.

5.2 Discussion

5.2.1 Physical site parameters

Measuring traces of oxygen *in situ* in water saturated sediments is highly sophisticated, as long as oxygen should no be consumed by measuring devices (Matthiesen 2005). Hence two systems were used to monitor the oxygen concentrations in the field, optical oxygen sensors for direct determination and additionally redox potential measurements as an indirect approach. However, conducted oxygen measurements in water saturated sediment had technical constrains. The reproducibility of the measurements in the sediment was very dependent on the air tight sensor installation. From the triplicate measurements at foundation height two sensors showed the same reading where as one deviated widely in case of the little bacterial decayed (LBD) site or slightly and then joining the other two readings in case of the severe bacterial decayed (SBD) site. Nevertheless, towards the end of the observation time nearly all oxygen sensors measured zero oxygen. The presented oxygen measurements in sediments surrounding wooden pile foundations indicate conditions with no free oxygen at both sites.

Oxygen can thermodynamically exist from a redox potential of 220 mV upwards and aerobic respiration can be found at a redox potential range between 450 to 800 mV (Schachtschabel et al. 1992). At both sites redox readings were most of the time around -200 mV. Hence oxygen free conditions were confirmed by redox potential measurements. However, at the SBD site redox potential 0.3 m above the pile head increased to +400 mV at two incidences which does not theoretically rule out the existence of oxygen, although it was not measured. This is possible as oxygen can exist from a thermodynamic point of view and possibly be causing the increase in redox potential. It is very likely that the penetrating oxygen is rapidly consumed, as anoxic environments are ample supplied with reduced components which are quickly oxidised upon availability of oxygen.

The presented redox potential measurements are from single measurements only although redox measurements are highly variable and best measured in triplicate (Mansfeldt 2003). Kölling (1986) reported under optimal conditions an error margin of ±50 mV. Therefore, the emphasis should be given to redox ranges rather than single values. There is some concern in the literature (Gregory 1998) that the interaction of the platinum with hydrogen sulphide under aqueous conditions could contaminate the electrode and provide false readings. As

further problems associated with permanent redox potential measurements was the contamination of the platinum electrode surface by reaction with oxygen and subsequent formation of platinum oxide. The measured potential is influenced by the oxide layer on the electrode surface (Fielder 2000).

The increase in the redox potential of the shallowest sensor at SBD site is preceded by low ground water table readings but does not occur in Mai and June 04 were equally low ground water tables were measured. Ground water table fluctuation was identified as the most important factor influencing redox potentials in redoximorphic soils followed by temperature and precipitation (Fiedler 2000). July 04 experienced heavy rain fall (Tab. 5.4) which might be the cause for the slight increase of the redox signal of the shallowest sensor at the LBD site in August 04. The infiltrating rain water transports oxygen towards the ground water (Wiechmann 1979), but considerable quantities of rain water are needed to influence ground water composition. The slight redox potential increase of the shallowest sensor at the LBD site in winter 04 could as well be explained with heavy rain, as from Dec 03 to Feb 04, 299 mm precipitation occurred (average for that period 192 mm, Tab. 5.4). It is interesting to note that the heavy rain fall periods in winter 03 and summer 04 only affected the redox readings at one of the two sites at a time. Ground water table fluctuations did not alter the redox potential around the pile head at the LBD site significantly in comparison to the SBD site. The reason is the larger distance between pile head and mean ground water table at the LBD site compared to the SBD site.

The precipitation in the measurement period (Tab. 5.4) was from December 2003 to February 2004 with 299 mm much higher as the average precipitation in that period with 192 mm. The same was observed from July 04 to August 04, when it rained nearly twice as much (249 mm) as normal (128 mm). In contrast March 04 to May 04 was dryer (108 mm) compared to the long term average year (172 mm).

The annual precipitation for the measurement period was 100 mm higher as the average annual precipitation. The ground water table fluctuation, measured at this exemplary year, did not give evidence of foundation exposure to unsaturated conditions at the LBD site. Nevertheless, the ground water table was lowered at the SBD site in September 03 to -0.72 m NAP which is 0.11 m under pile head level of -0.61 m NAP. At the SBD site head of foundation exposure due to very dry weather was recorded.

Tab. 5.4: Precipitation during the measurement period together with the long-term average precipitation per month at the measurement station De Bilt close to Amsterdam (Royal Netherlands Meteorological Institute 2004).

	2003							2004									
Month	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	Oct-03 - Sep-04
Precip. [mm]	35	30	10	52	84	40	96	123	79	42	33	31	69	122	127	54	901
Average Precip. [mm]	*72*	*70*	*58*	*72*	*77*	*81*	*77*	*67*	*48*	*65*	*45*	*62*	*72*	*70*	*58*	*72*	803

Additionally the temperature was determined together with the oxygen measurements. The observed difference between the two sites could mirror the installation depth differences of 0.5 m between the sites. The mean temperature is slightly higher than the mean annual temperature for the region because of the proximity of the sites to houses, its pavement and the shallow measuring depth. In general bacterial activity increases with increasing temperature. Therefore, higher temperatures might have a favouring influence on bacterial wood decay. Otherwise the bacterial community is likely to alter with changing temperature which complicates simple statements.

The ground water flow at the two sites does not differ much. Therefore, the assumption that the severely degraded site has a higher ground water flow can not be supported. In contrast the little decayed site has a slightly higher flow than the severe one. Nevertheless, both sites do vary in the ground water flow direction. At the severe decayed site the flow is towards the Sapharti Park which was expected from piezometric ground water table measurements.

5.2.2 Chemical site parameters

The sediment water composition between the two sites differed in the total nitrogen concentration with higher values at the LBD. The analysed inorganic nitrogen was at both sites mostly in the form of nitrate. The presence of nitrate and the measured low redox potentials are contradictory. However, the basic pH and relatively high DOC concentrations

in the sediment solutions from both sites might have triggered nitrate formation after sampling. Therefore, it can not be ruled out, that the measured nitrate was a sampling or storage artefact. Nevertheless, total N concentration would not be affected and that was analysed to be highest at the LBD site.

The sediment water composition between the two sites differed also in the sulphate concentration with higher values at the SBD. The sulphate concentration was very variable between samplings and showed a marked depth gradient. In the solution only sulphur was analysed. Therefore, sulphate values should not be over interpreted. However, higher sulphur concentrations were also found in the sediment at the SBD site.

In the sediment is the slightly higher carbon concentration at the LBD compared to the SBD site probably influencing the also higher sulphur and calcium concentrations. Sulphur and calcium in sediments are often associated with organic matter. However, the sulphur concentration difference between the two sites is much higher as the carbon one. Focusing on the sediment solution composition the sulphate variation at the SBD site can not be explained by DOC variance and is more likely caused by differing sulphate ion concentration in the ground water stream at the SBD site.

From the chemical analysis of the sediment solution and the ground water flow direction measurements it could be assumed that both sites are supplied from ground water with different compositions. In the ground water at the LBS site more sodium and chloride are present. whereas at the SBD site calcium and sulphate prevail.

At both sites the sediment pH is basic (7.6) which is mirrored also in the sediment water pH (7.9). Basic conditions were also found at two other sampling sites in Amsterdam (Chapter 4). Anoxic systems are well buffered in a basic pH range (Jordan 2001), however, basic sediment conditions can also be caused by natural chalk contents and building materials introduced to the sediment during house construction. In the sediment solution most (70-90%) of the total carbon was inorganic which is not unusual at this pH levels.

5.2.3 Conclusions

The results presented from the long-term measurements suggest that oxygen concentration and ground water flow velocity can not explain decay intensity differences at the two sites. It appears that the distance of the pile head to the average ground water level is the best explanation for the bacterial wood decay differences at the investigated sites. The shallower foundation depth at the severely degraded site can account for the lower carbon and nitrogen concentrations found at this site. As both sites have similar sediment profiles, the deeper foundation is situated deeper in the sandfill and is lying closer to the underlying peat. It was not possible to sample the peat underneath the two measurement sites, therefore, it can only be speculated that the peat is the nutrient rich *carex* sp. peat. *Carex* peat is often found in Haarlem which is west of Amsteram, whereas *Spagnum* peat, which is nutrient poor, exist north and south of Amsterdam (Abrami et al. 2005). Transport of nitrogen and carbon rich ground water from the peat layer upwards could supply the sandy sediment at the deeper foundation site with carbon and nitrogen. Although the general ground water flow direction is downwards in the parts of Amsterdam built before 1940 (Herman Keijer personal communication) it is possible that in dry summer months the peat layer liberates some water to the overlaying sand layer to equilibrate different water pressures. Additionally, diffusion of dissolved carbon and nitrogen is also a possibility as the relevant time scale is over hundred years.

6 Studying bacterial wood decay under low oxygen conditions – results of microcosm (MC) experiments

6.1 Results

6.1.1 Different gas supplies (MC experiment I)

6.1.1.1 Oxygen profile in the microcosms (MCs)

Fig. 6.1 shows the oxygen profile in the MCs resulting from the different gas supply treatments. In the nitrogen treatment, no oxygen was detectable at all depths. For the other treatments a steep gradient in oxygen concentrations was recorded at the sediment-water interface corresponding to the solubility of oxygen in the overlaying water down to zero at a 1 to 2 cm depth in the sediment were measured.

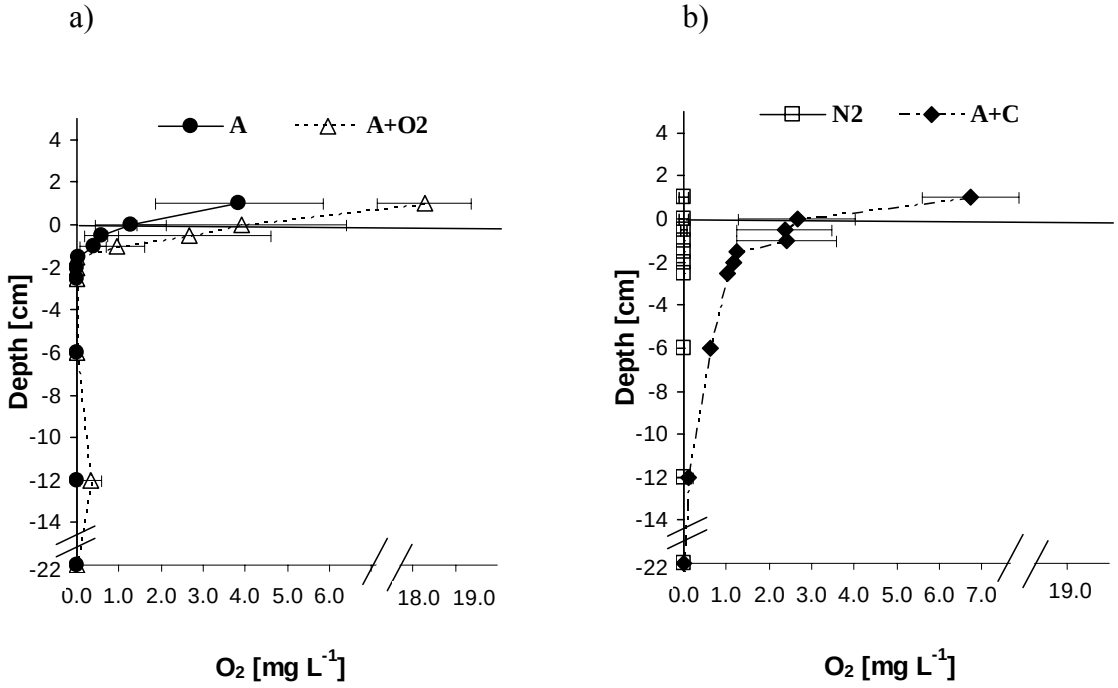

Fig. 6.1: Mean (±SE) vertical gradient of oxygen concentration in the MC over 400 days, a) A = Air, A+O_2 = Air enriched with Oxygen, b) N_2 = Nitrogen, A+C = Air and water circulation. Positive values are within the overlaying water, negative values within the sediment core, n = 4 for depth +1.0, 0, -0.5, -1.0, -12 cm and n = 1 for depth -1.5, -2.0, -2.5, -6.0, -22.0 cm. Note: The O_2 value in the overlying water for A+O_2 is 18.32 mg L^{-1} and the last measured depth is -22.0 cm.

The air and water circulation treatment (A+C) resulted in oxygen being measured further down in the sediment. Even at 6 cm depth in the sediment about 1 mg L^{-1} oxygen occurred. It was noted that the reproducibility of the oxygen concentration between the four MCs of one treatment was low.

Fig. 6.2 shows the development of the oxygen concentration in MCs of the A+C (air and circulation) treatment at five different depths during the time course of the experiment. The standard error is not shown for clarity reasons. The mean oxygen concentration is 1.34 mg L^{-1} with values ranging from min = 0 mg L^{-1} to max = 2.45 mg L^{-1}. The highest oxygen concentration was always measured in the water overlying the sediment. Before the start of the water circulation, oxygen saturation (18°C) occurs in the overlying water. After the onset of the water circulation the oxygen concentration decreased and then increased again after five months. At the start of the experiment only at the sediment water interface was oxygen detectable. After two months oxygen was measured at a depth of 1 cm. After six months oxygen was even present 12 cm deep into the sediment. These measurements show that oxygen penetrated deeply into the sediment during the course of the experiment.

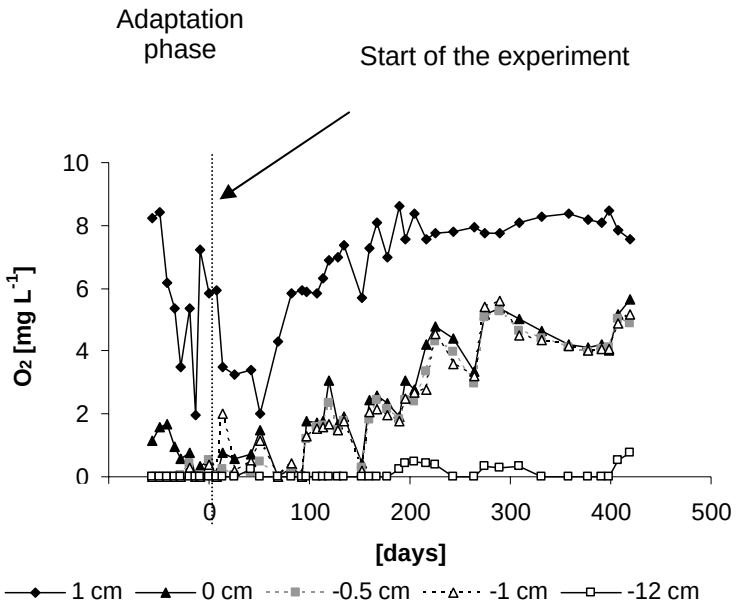

Fig. 6.2: Mean oxygen concentration in the air and circulation (A+C) treatment at different depth during the experiment (n = 4, 0 = start of the experiment with water circulation and gas measurements, SE not shown for clearness of the picture).

6.1.1.2 Bacterial decay intensity

In all treatments bacterial wood attack was found (Fig. 6.3). From 120 to 250 days decay intensity increased with incubation time whereas afterwards (400 days) no substantial increase in decay intensity was observed. However, the oxygen enriched air treatment ($A+O_2$) shows nearly constant values with increasing incubation time. Bacterial decay intensity in the nitrogen (N_2) and air with circulation (A+C) treatments appears to increase steadily with incubation time. In the oxygen-enriched treatment, bacterial wood decay was first observed after 150 days and from 195 days onwards showed comparable high intensities. In all samplings carried out, the highest observed bacterial decay intensity was found in the air treatment with water circulation. However in this treatment soft rot attack was noticed in wood from the upper layer in the microcosms after 195 days (Fig. 6.4 and Fig. 6.5).

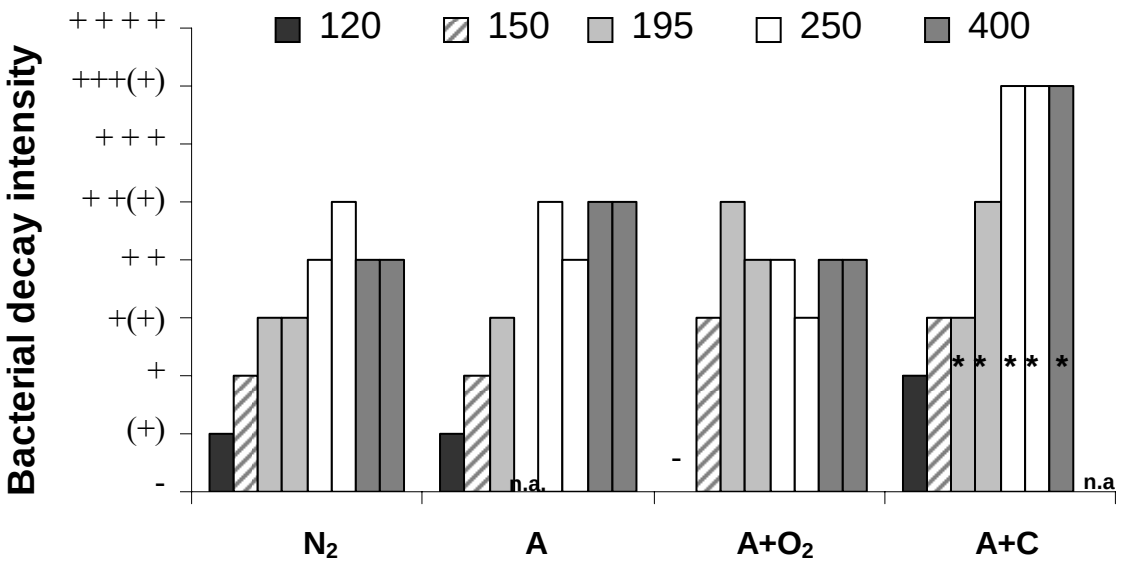

Fig. 6.3: Intensity of bacterial decay (number of crosses) in the four microcosm treatments after different incubation times (in days), n.a. = not analysed, * = soft rot decay, "-" = no bacterial decay, for treatment abbreviations see Fig. 6.1. (from Kretschmar et al. 2007a)

The degree of bacterial wood decay was assigned after inspection of samples from different locations in the MCs. The investigations showed no differences in the bacterial attack

between samples of the inner and outer circles. Nevertheless, in all treatments differences between the upper and the lower layer could be observed (not shown). In these cases the degree of attack was always higher in the upper layer than in the lower one. The difference in attack between upper and lower layer degreased in the A+C treatment with incubation time, whereas in all other treatments the difference prevailed.

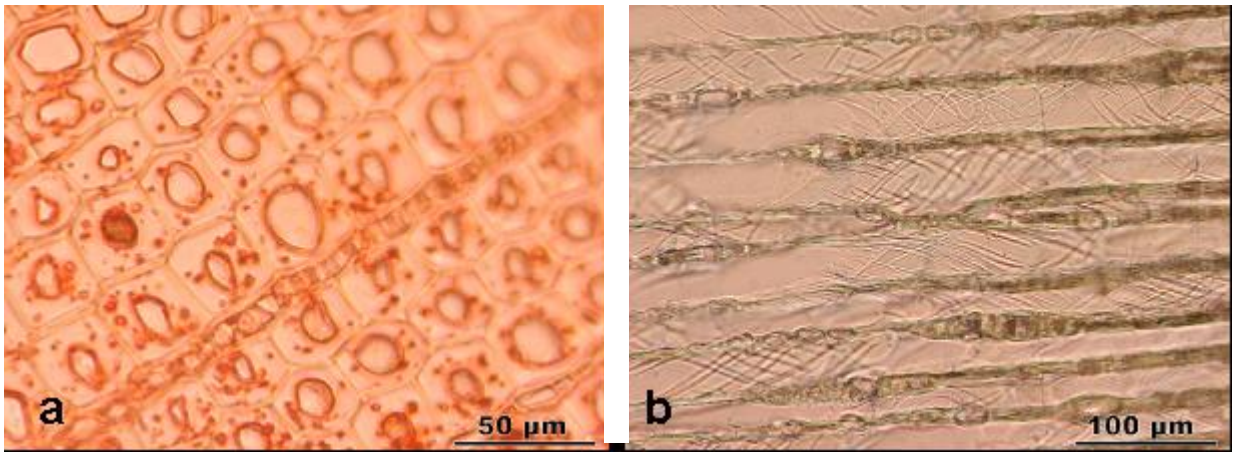

Fig. 6.4a, b: Soft rot attack in the air and water circulation treatment after 195 days **a)** transverse section **b)** longitudinal section (from Kretschmar et al. 2007a).

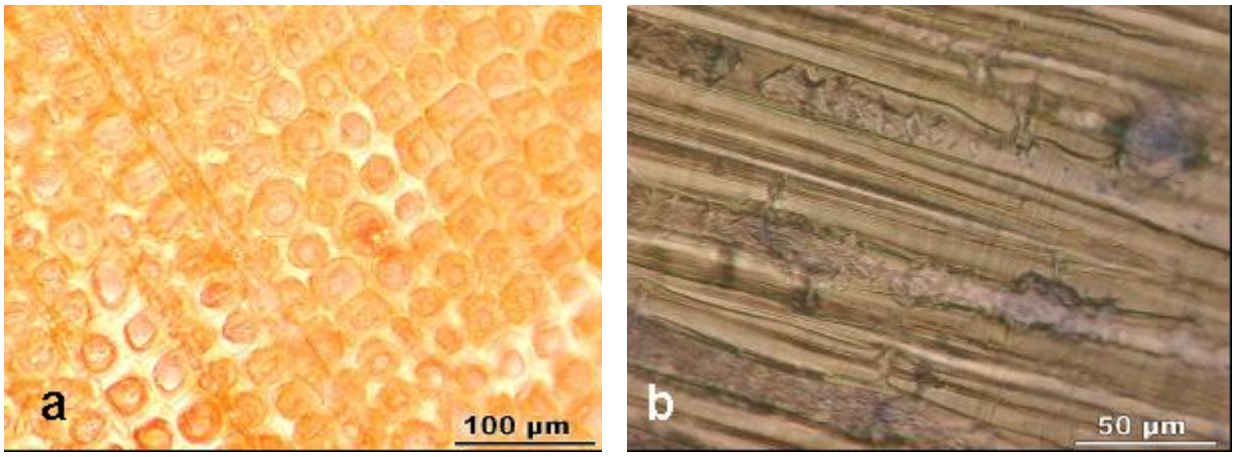

Fig. 6.5a, b: Heaviest bacterial attack of pine sapwood in the water circulated treatment after 350 days. **a)** In transverse section nearly all cells are completely degraded, **b)** in this stage of attack the characteristic grooves and cavities of bacterial degradation can be observed in the longitudinal section (from Kretschmar et al. 2007a).

The simultaneous occurrence of decay patterns by soft rot fungi and erosion bacteria in one section is shown in Fig. 6.6. However, this is no evidence that both wood degraders are active under the same environmental conditions. Soft rot was not detected prior to 195 days incubation time, whereas erosion bacteria (EB) decay patterns were observed from 120 days onwards. Thus it is possible that EB start degrading the wood but are joined by soft rot fungi after a subsequent change of environmental conditions (e.g. oxygen content). Whether erosion bacteria continue wood degradation under environmental conditions favouring fungal decay is unknown.

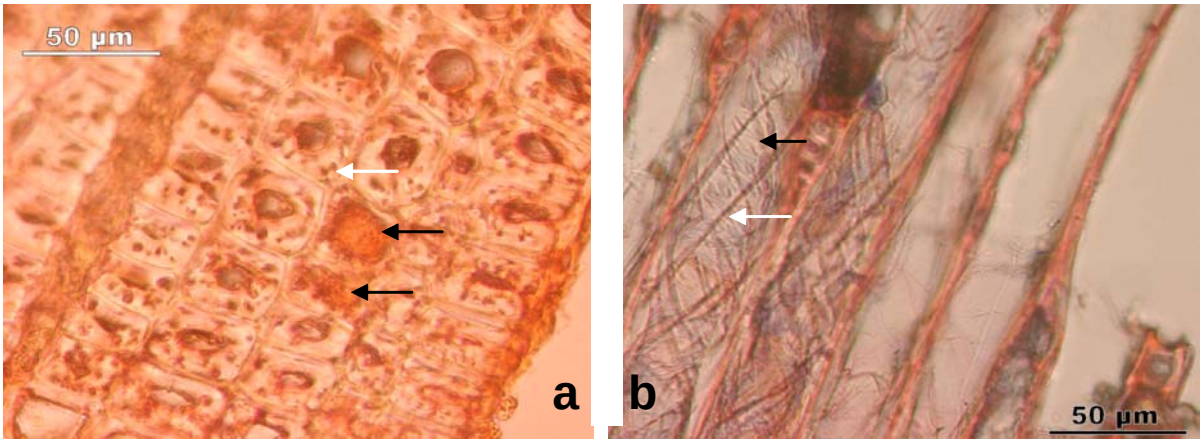

Fig. 6.6a, b: Simultaneous occurrence of erosion bacteria (black arrows) and soft rot fungi (white arrows) decay patterns in **a)** transverse section and **b)** longitudinal section (from Kretschmar et al. 2007a)

6.1.1.3 Gas production

Mean CO_2-C production for all microcosms per treatment as an average over all replicates is shown in Fig. 6.7a, b . The production differs between the different gas supply treatments.

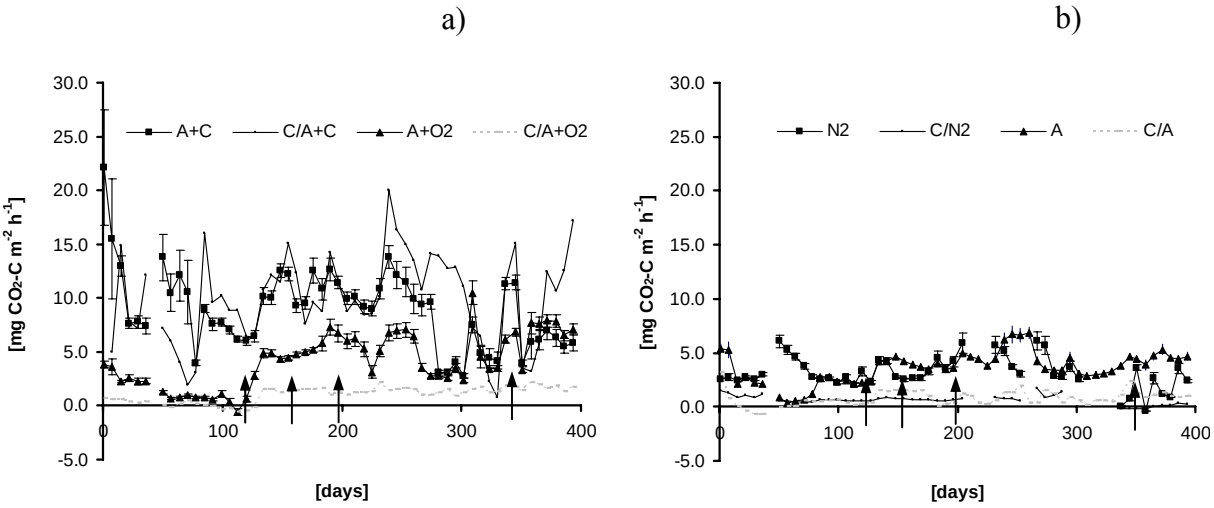

Fig. 6.7a, b: Mean (±SE) CO_2-C production in the different gassing treatments (weekly average). Arrows indicate sampling times and consequently change in n, Treatments: n=12 at 0 d to 120d, n=11 at 120 d to 150 d, n=10 at 150 d to 195 d, n=8 at 195 d to 350 d, n=6 at 350 d to 400 d, Control Air (C/A) n=3, Control air and water circulation (C/A+C), air and oxygen (C/A+O_2) and nitrogen (C/N_2) n=1).

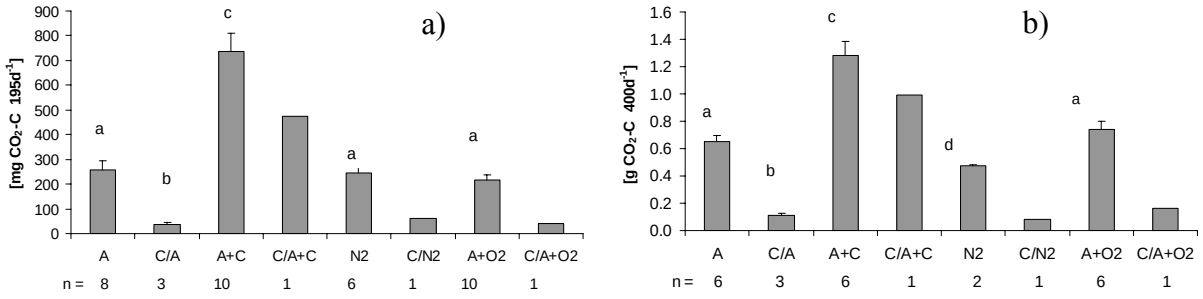

Fig. 6.8a, b: Mean (±SE) cumulative CO_2 emission for different MC treatments, **a)** after 195 days in mg CO_2-C, **b)** after 400 days in g CO_2-C, n = number of replicates given below treatments. Different letters indicate significant differences between treatments at $\alpha<0.05$. For treatment abbreviations see Fig. 6.1.

The control (without wood) of all treatments shows, with some exceptions, lower CO_2-C production than the corresponding experimental treatment. The A+C treatment displays the highest CO_2-C production, whereas the difference between the other treatments is not so pronounced. The CO_2-C emission varied considerably between the replicates and with time. No pattern was found for the variation with time, therefore, no trend analysis was performed.

Fig. 6.8a shows the cumulative CO_2-C emission for the different experimental treatments and the corresponding controls after 195 days. There is no significant difference between the Air treatment and the A+O_2 and N_2 treatments. Since the controls for the A+C, N_2 and A+O_2 treatment had no replicates, it was not possible to verify statistically differences between the treatment and their corresponding control. After 400 days (Fig. 6.8b) the CO_2 emission of all treatments is significantly different. The highest wood effect, i.e. the difference between the experimental treatments with wood and the control without wood, on the CO_2 emission occurs with the A and A+O_2 treatments.

6.1.2 Chemical sediment composition (MC experiment II)

6.1.2.1 Bacterial wood decay intensities

Using the evaluation scheme described in the MC experiment I, only slight or no bacterial attack could have been assigned in MC experiment II (Tab. 6.1). Treatments with pure sediment (S), silica sand (SS) or their mixture (M) show very slight bacterial wood decay in most of the samples. Furthermore, treatments in which phosphorus (PO_4) and sulphur (SO_4) were added to the sediment, did not show any sign of attack after 155 days. However in one single MC of the S+P (added PO_4) and the S+A (added ammonium) treatments some very slight signs of bacterial attack were observed.

Tab. 6.1: Intensity of bacterial decay in different treatments (S = sediment, M = mixture from sediment and silica sand (SS), S+A = S with ammonium addition, S+N = S with nitrate addition, N+P = S with phosphate addition, S+Su = S with sulphate addition) in the MC experiment II, - = no signs of bacterial wood decay found, (+) = very slight decay, + = slight decay (from Kretschmar et al. 2007a)

Treatment / Parallels	S	M	SS	S+A	S+N	S+P	S+Su
1	(+)	(+)	(+)	(+)	-	(+)	-
2	(+)	(+)	(+)	-	-	-	-
3	(+)	(+)	(+)	-	-	-	-
4	(+)	(+)	+	-	-	-	-

In the second stage of the microcosm experiment the evidence of bacterial attack was mostly in single wood cells (Fig. 6.9). Attacked neighbouring cells were scarcely observed.

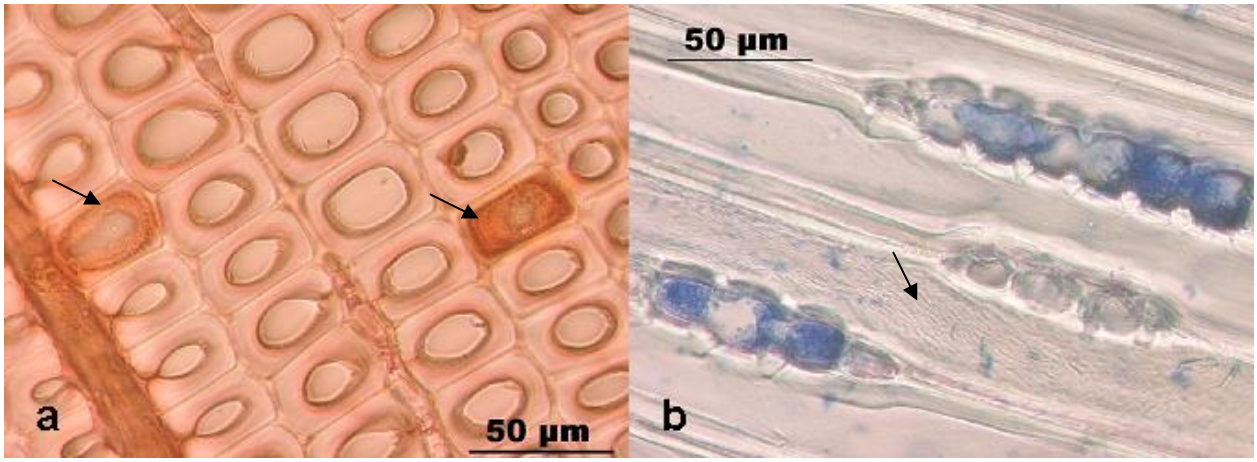

Fig. 6.9a, b: Examples of bacterial decay (arrowed) most often observed in the second MC experiment II **a)** transverse section **b)** longitudinal section, (from Kretschmar et al. 2007a)

6.1.2.2 Fungal wood decay

During the experiment gas was produced in the MC which resulted sometimes in wood samples being pushed out of the sediment. Wood exposure to aerobic conditions in the overlaying water resulted in fungal decay (Fig. 6.10).

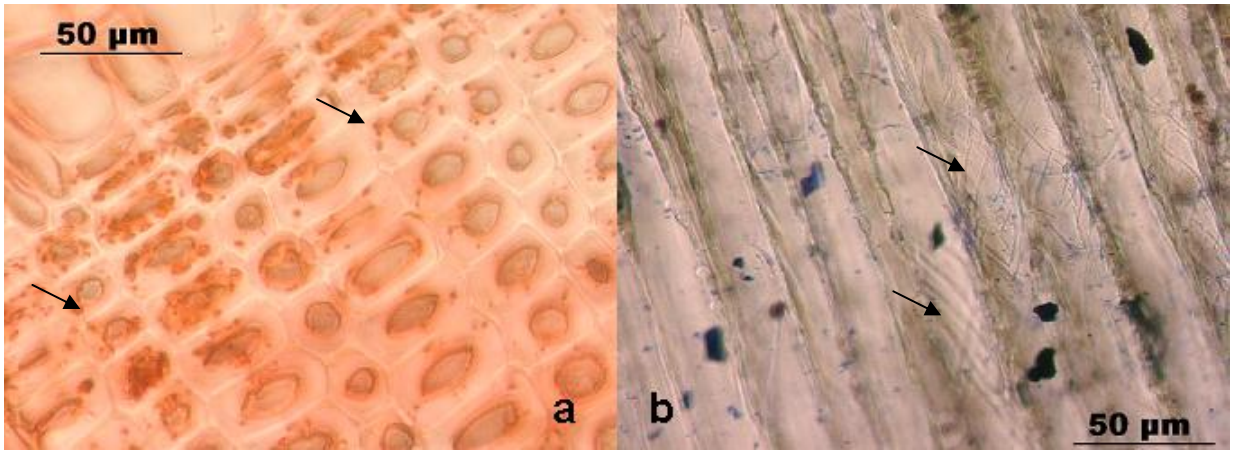

Fig. 6.10a, b: Typical soft rot decay in **a)** transverse and **b)** longitudinal section showing cavities (arrowed) in axial tracheids of pine. (from Kretschmar et al. 2007a)

6.1.2.3 Chemical sediment composition

The "dilution" of the sediment with silica sand in the mixture (M) treatment was successful as the sediment carbon concentration (Tab. 6.2) in the S, M and SS treatment illustrates. However, as anticipated the phosphate and sulphate concentrations are less reduced. The nitrogen addition to the sediment was as expected (~1.0 mg g^{-1}) in the form of nitrate (S+N), whereas in the form of ammonia (S+A) less than desired (~0.6 mg g^{-1}) was added.

Unfortunately no adjustment of sediment pH was conducted after the chemical addition. The basic sediment pH (8.3) was considerably raised by phosphorus addition (10.1) and slightly lowered by ammonia addition (7.4) at the beginning of the experiment. Silica sand (SS) had a very low pH of 5.4 at the beginning. However, sediment pH at the end of the experiment differed widely from the pH at the beginning in the different treatments. In summarising all the treatments, it can be observed that sediment pH was less extreme at the end compared to the start of the experiment. (Silica sand showed the same pH as pure sediment which is a

considerable increase of 2.6 pH units. Ammonia addition on the contrary lowered further the pH to 6.7, whereas the phosphorus addition resulted in a less basic pH at the end compared to the beginning.)

Tab. 6.2: Chemical sediment composition in the different treatments at the beginning of the experiment (total element concentrations) and sediment pH at the start and the end of the experiment, dl = detection limit (N = 0.1 mg g^{-1}), for treatment abbreviations see Tab. 6.1.

	C_t	N_t	P_t	S_t	pH start	pH end
Treatment	[mg g^{-1} DW]					
S	4.2	0.11	0.16	0.25	8.3	7.9
M	2.2	<dl	0.11	0.21	8.4	8.0
SS	0.0	<dl	0.07	0.17	5.4	8.0
S+A	4.6	0.62	0.19	0.38	7.4	6.7
S+N	4.3	0.96	0.18	0.42	8.2	8.9
S+P	3.9	0.12	0.48	0.20	10.1	9.3
S+Su	4.1	0.12	0.14	0.45	8.2	8.5

Bacterial wood decay intensity in this experiment appears to be highest at a sediment pH range from 7 to 8.3 in the treatments without chemical addition to the sediment (Fig. 6.11). The addition of chemicals to the sediment did affect the sediment pH and it can, therefore, not be distinguished between the pH effect and the chemical effect of the addition. Nevertheless, there exist two outliers out of four parallels with decay detected at low (6.5) and high (9.4) pH values.

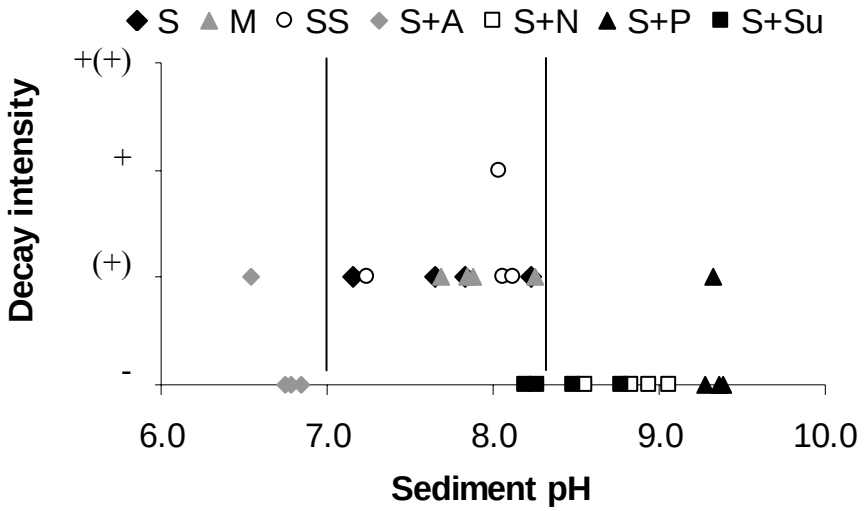

Fig. 6.11: Bacterial wood decay intensity versus sediment pH at the end of the experiment. Vertical lines confine pH range in which bacterial wood decay was mostly detected, for treatment abbreviations see Tab. 6.1.

6.1.2.4 CO_2 gas emission

For all treatments statistically different CO_2-C emissions (Fig. 6.12) were analysed compared to their corresponding controls without wood. In all treatments and in most of the controls CO_2 was emitted. Only the silica sand (SS) and pure sediment (S) controls absorbed CO_2. Mean values for the treatments with wood varied only slightly between the different treatments and are not statistically different. In the controls (no wood) and in the treatments with wood, the ammonium addition (S+A) resulted in the highest measured CO_2-C emission, followed by the phosphate addition (S+P) and the mixture treatment (M).

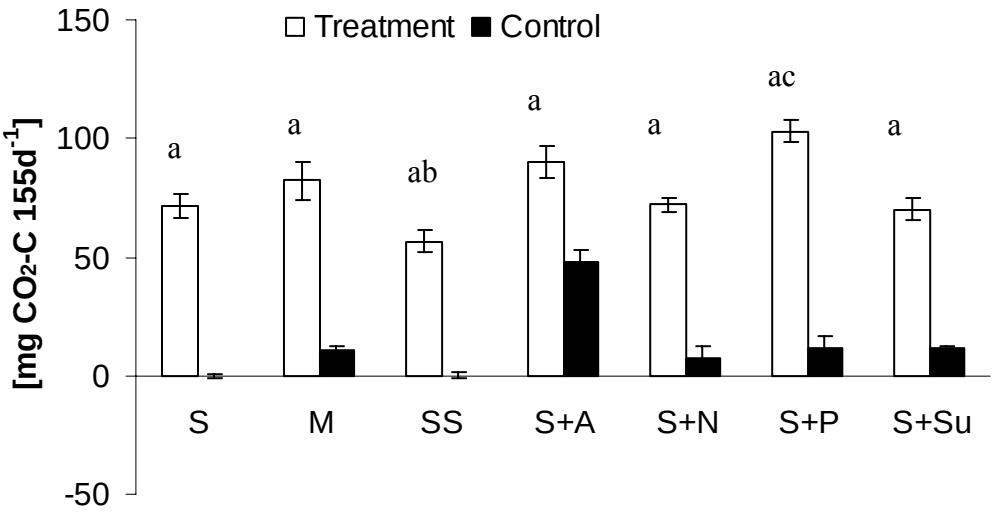

Fig. 6.12: Mean (±SE, n=4) cumulative CO_2-C emissions after 155 days of the different treatments (sediment + wood) and controls (no wood) for different sediment addition treatments. All treatments differ significantly ($p<0.05$) from their corresponding controls (not marked). Different letters indicate statistical difference at $\alpha=0.05$ between the different treatments. For the controls no significant differences were found between treatments, for treatment abbreviations see Tab. 6.1.

To assess the pure effect of wood addition to the CO_2-C production, experimental treatment emission rates were standardised by subtracting their corresponding control values (Fig. 6.13). Statistical analysis of the resulting wood derived CO_2-C production reveals that all treatments belong to the same population. However, significant differences ($p<0.05$) exist between the lowest value found in the S+A and the highest in the S+P treatment.

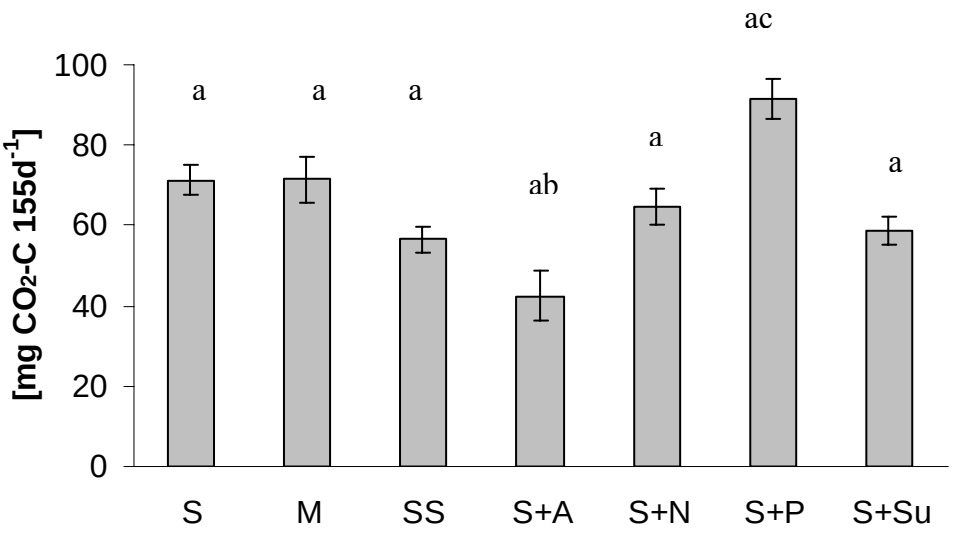

Fig. 6.13: Mean (±SE, n=4) cumulative wood derived CO_2-C emissions after 155 days. Different letters indicate statistical difference between treatments at α=0.05, for treatment abbreviations see Tab. 6.1.

6.1.2.5 CH_4 gas emission

MC cumulative CH_4-C gas emission does not show such a distinctive pattern compared to the CO_2-C emission (Fig. 6.14). Only the experimental treatments (sediment with wood addition) with sediment (S), silica sand (SS) and their mixture (M) showed CH_4 emissions, whereas the other experimental treatments and all controls (sediment without wood addition) absorbed CH_4 from the internal gaseous atmosphere (aeration).

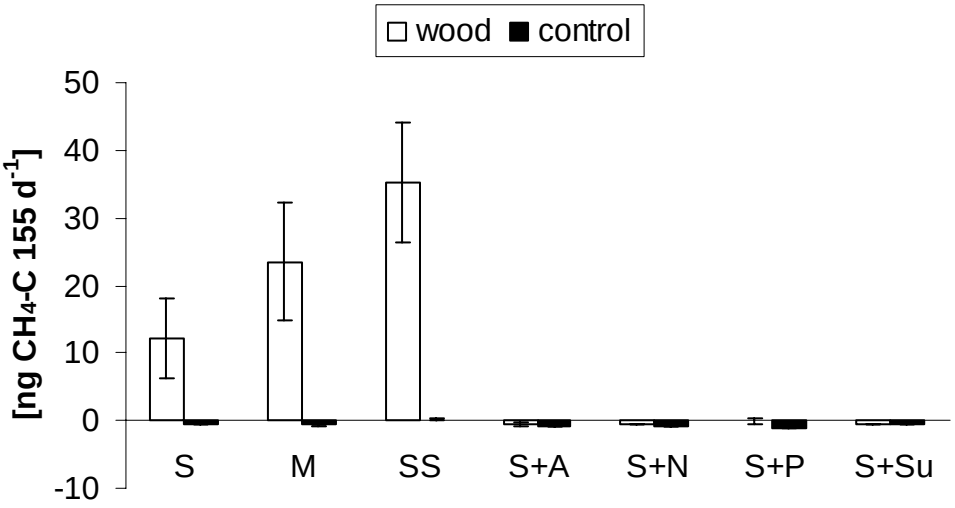

Fig. 6.14: Mean (±SE, n=4) cumulative CH_4-C emissions after 155 days of the different treatments and their corresponding controls (without wood addition). The control differs statistically from the corresponding treatment at α=0.1 in all treatments, for treatment abbreviations see Tab. 6.1.

The experimental treatments with chemical addition to the sediment resulted in higher CH_4-C absorption in the corresponding controls than in the treatment itself. Cumulative CH_4-C absorption shows a high SE due to discontinuous methane release in bubbles.

6.1.3 Prevention method (MC experiment III)

6.1.3.1 Gas production

In the MC sediment with glucose addition methane was produced vigorously. The concentrations were outside the GC measuring range and caused, together with water, a system failure. Therefore, no record of the CO_2 and CH_4 production exists.

The methane could not escape immediately from the sediment and accumulated within it. Hence, the sediment volume increased considerably, which reduced the MC headspace. In all MCs with glucose addition, water was removed to prevent it from entering the GC-system. Later on, in some MCs the water overlying the sediment filled space in the sediment formerly

occupied by released gas. This caused the sediment surface to dry out which was noted in some cases at the end of the incubation time during kapok bag removal.

6.1.3.2 Kapok decay intensity

The chemical addition treatments of the third experiment consist of the same four different gassing treatments employed in the first experiment. In the control treatment serious bacterial degradation (+++) took place in kapok (Fig. 6.15). The addition of glucose resulted in less bacterial decay of kapok, whereas in some MCs kapok was degraded by fungi. The addition of glucose and sulphate resulted in almost no bacterial decay of kapok. The fungal decay was caused by soft rot and was found in four MCs from the water circulation (A+C) treatment and also in one from the air treatment (A). The extent of decay of kapok fibres removed from the bags in any one MC was generally similar.

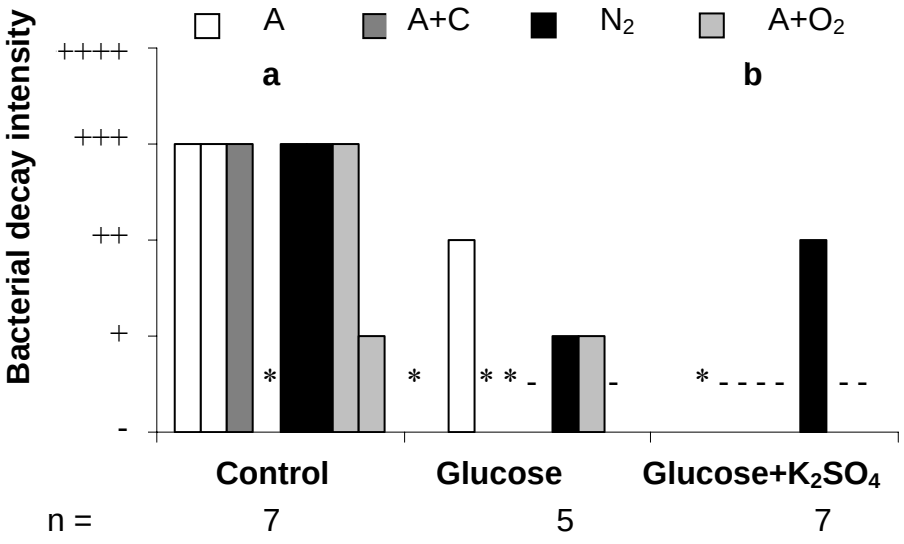

Fig. 6.15:Median kapok decay per MC (n per MC varying between 1 to 4, n per treatment stated, depending on number of failures through fungal decay) - = zero bacterial attack, * = fungal decay. Different letters indicate statistical difference at ($\alpha<0.05$).

The median kapok decay intensity in the glucose and sulphate addition treatment differs statistically from the control. However glucose-only addition to sediment does not result in significant kapok decay intensity difference compared to the control and the other addition treatments.

6.2 Discussion

6.2.1 Different gas supplies (MC experiment I)

Bacterial attack of wood specimens was detected in all treatments regardless of different gas supplies to water overlying sediment with air (A), oxygen enriched air (A+O_2) or nitrogen gas (N_2). Only the initial stages of bacterial decay were observed, but decay was strongest in the air with water circulation (A+C) treatment. No oxygen was measured in all sediments without water circulation. Only in the water circulated and air enriched treatment was oxygen detectable in the upper parts of the sediment, but this only migrated further down into the sediment profile in the air and water circulation treatment.

The oxygen concentration in the microcosms could be monitored successfully with oxygen optodes and recorded for instance the expected steep oxygen concentration gradient at the sediment-water interface. The small variation of zero oxygen concentration in the different gassing treatments (air, oxygen enriched air, nitrogen gas, air and water circulation) at the start of the experiment also corresponded to expectations.

However, the installation of the optodes with silicone rubber tubing gaskets caused some atmospheric oxygen penetration into the MCs. In the nitrogen treatment this resulted in significantly higher CO_2-C production rates in MCs with optodes compared to MCs without optodes. Nevertheless, this effect was not significant in the other treatments because of oxygen availability in the air inflow. This probably resulted in CH_4 conversion to CO_2 in the water overlying sediments, which masked any effect of oxygen intrusion from the optode installation. It is very likely that the additional oxygen supply from the optode installation had no effect on the overall oxygen concentration as anoxic conditions prevailed in three out of four microcosm treatments. In anoxic conditions oxygen is rapidly consumed by oxidation of reduced components and oxygen contact with wood is prevented. Methane was emitted in all treatments despite an oxygen supply to water overlying the sediments which indicates that reduced conditions prevailed in the sediment. Therefore we conclude that although installation of oxygen optodes altered the oxygen supply to the microcosms the oxygen measurements can be regarded as reliable.

At the beginning of the experiment, easily degradable organic matter breakdown utilised oxygen and prevented deeper oxygen penetration into the sediment. Upon depletion of the readily degradable substrate the breakdown of organic matter decelerated. With less oxygen

needed for organic matter breakdown in the upper sediment horizonts, it was apparent that oxygen diffused into deeper sediment layers and was measurable at one centimetre depth in the A+C treatment after approx. 100 days.

Bacterial decay in wood specimens was determined by light microscopic investigation. All the decayed areas were in the initial stage of attack which is not surprising given the relatively short experimental duration of 400 days. Björdal and Nilsson (2005) used thin wood section to study wood decaying bacteria over short incubation times and also recorded the occurrence of bacterial decay with light microscopy. Bacterial wood decay classification schemes based on light microscopic investigations exist for foundation pile investigations (Klaassen 2005) or archaeological wood, but due to much longer time spans for exposed field specimens, bacterial decay is more intense and the classification scheme did not serve our purposes. The newly developed classification scheme presented here for initial bacterial wood decay is a synthesis of the classification idea from Klaassen (2005) and the description of initial decay by Björdal and Nilsson (2005).

Daniel and Nilsson (1986) used Transmission Electron Microscope (TEM) images to document erosion bacterial decay progression on a single wood cell basis. Kohlmeyer (1978) used light microscopy to detect the occurrence of initial bacterial decay but no classification was described. Light microscopy was mostly used for bacterial wood decay classification when more severe degrees of decay were observed (Paajanen and Viitanen 1988; Grinda 1997; Klaassen 2007a). These authors used four to five degree classes where the degree described here is roughly from class zero to a maximum of 2. However, degree classification from single wood sections is impossible because of non-homogeneous decay distribution in wood. Apparently sound wood fibres are found among heavily degraded ones forming a chequered pattern in transverse sections (Blanchette et al. 1990). Nevertheless, recent investigations showed that the pattern of apparently sound wood cells directly adjacent to decayed ones is only due to the 2-D technique applied. By using 3-D images, gradients of decay in tracheids ranging from sound to moderately decayed areas were revealed (Björdal et al. 2005).

CO_2 emissions in the microcosms were measured for two reasons: First, to verify if the CO_2 emission could be taken as an indicator for the decay process and second, to derive a carbon budget together with CH_4. However, applying CO_2 measurements in the head space of the microcosms was not done to elucidate any insight into ongoing CO_2 -producing processes.

CO_2 emissions might have several sources such as aerobic organic matter breakdown or anaerobic degradation which liberates CO_2 in different proportions.

The cumulative CO_2-C production showed little variation between the treatments which is also true for the bacterial wood decay intensity except in the case of the aeration and water circulation treatment. Fig. 6.16 shows the correlation between the mean wood derived CO_2-C emission rate and bacterial decay intensity for the A+C treatment. Only a weak correlation of r^2=0.47 was calculated. In contrast no correlation was evident for the other treatments.

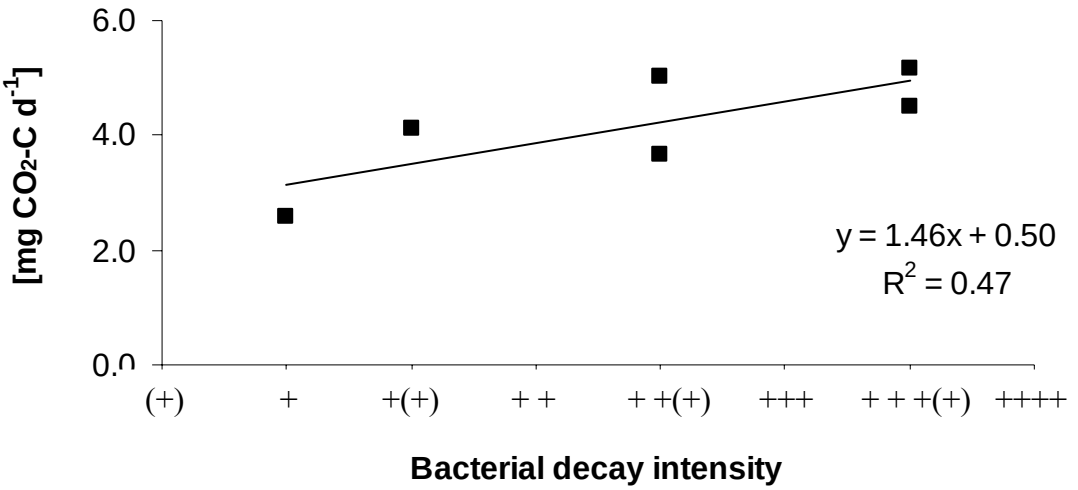

Fig. 6.16: Mean CO_2-C emission of the air and water circulation treatment (A+C) versus bacterial decay intensity

As no correlation existed for the other treatments the indicator function of CO_2-C production for bacterial wood decay intensity seems to be questionable. Moreover the N_2O and CH_4 emissions could not be related to bacterial wood decay intensity. In the case of CH_4 this could be due to two processes: first, the conversion of CH_4 to CO_2 in the water overlying the sediment in treatments with aeration, second, the inconstant release of methane from the sediment by bubbles. Therefore, the time course of the methane release is very variable and difficult to measure with only four measurements a day. The air and water-circulation treatment prevented gas accumulation within the sediment presumably through enhanced gaseous exchange via preferential water flow channels. Therefore, it was only in this

treatment that an indication of CO_2 emission corresponding to bacterial wood decay intensity, although only a weak one, was seen.

In the oxygen-enriched air treatment (A+O_2), a marked increase in the CO_2 production coincided with deeper O_2 penetration into the sediment. The increase in the bacterial wood decay intensity with increasing CO_2-C production rate in the A+C treatment could be explained by an increased oxygen penetration and hence increased CO_2 emission and increased bacterial wood decay. It might be suggested that under enhanced oxygen availability bacterial wood decay proceeds faster. The other treatments (A, A+O_2 and N_2) showed relatively lower bacterial wood decay intensities which could be related to the limited availability of oxygen. It might be suggested as well, that either the N_2 treatment did have sufficient oxygen availability or that bacterial wood decay can proceed without free oxygen present.

In the circulated-water treatment soft rot decay was found because of the relative high oxygen values in the upper sediment layers. In Fig. 6.6a, b decay patterns of soft rot fungi and erosion bacteria can be seen. However, that does not prove that both wood degraders are active at the same time. Erosion bacteria were detected after 120 days, whereas soft rot fungi were not noted before 195 days. Erosion bacteria appear to predominate in environments where oxygen is limited (Nilsson and Daniel 1983; Daniel and Nilsson 1986; Blanchette et al. 1990; Singh et al. 1990; Singh and Butcher 1991; Nilsson and Daniel 1992).

A possibility is that erosion bacteria were the first wood degraders before soft rot fungi appeared and this occurred after a change in the environmental condition. The oxygen concentration in one centimetre depth of the sediment rose in the circulation treatment (A+C) after 100 to 120 days to 1 to 2 mg L^{-1} oxygen thus enabling growth of soft rot fungi. Oxygen levels of less then 0.03 mmol L^{-1} (0.48 mg L^{-1}) were reported to prevent fungal growth under laboratory conditions (Kohlmeyer and Kohlmeyer 1979). After 350 days incubation, soft rot patterns were detected without any signs of erosion bacterial decay patterns. Because soft rot fungi are faster wood degraders than erosion bacteria, it is possible that the erosion bacteria were out-competed by the soft rot fungi or more likely that bacterial erosion patterns were obscured by soft rot decay patterns. Only when environmental conditions result in waterlogging which is linked to poor oxygen availability or the presence of fungicides detrimental to fungi, are bacteria the main microbial wood degraders (Eaton and Hale 1993).

DNA extraction from MC water (results not shown) recovered from the A+C and A treatments proved to be isolates from the genera *Bacillus sp.* and *Sphingomonas sp.*. Unfortunately, DNA extraction was not possible for all treatments probably because of very low concentrations. In general bacterial wood decay has been discussed as a complex system with active primary wood-degrading bacteria and secondary bacteria living from the fragments of wood decay (Greaves 1971). Therefore the presence of bacteria in decayed wood does not necessarily imply that they are primary wood degraders. However, the secondary colonizers can have an influence on the population of primary wood degraders through antagonistic activities.

It can be concluded from the findings that bacterial wood decay can proceed without free oxygen present but that it is more intense if oxygen is available. However, only differences in the velocity of bacterial wood decay were observed, resulting in differences of decay intensity. Under the investigated conditions no differences in the general occurrence of bacterial wood decay were evident. The same was observed in field samples (Huisman et al. 2007b; Chapter 4, this work) where bacterial wood decay was found in a multitude of environmental conditions.

We conclude from our findings that bacterial wood decay can proceed without free oxygen present but that it is more intense if oxygen is available. However, it must be remembered that we only observed differences in the velocity of bacterial wood decay resulting in differences of decay intensity. Under the investigating conditions no differences in the general occurrence of bacterial wood decay could be observed. The same was observed in field samples (Huisman et al. 2007a; Huisman et al. 2007b; Chapter 4, this work) where bacterial wood decay was found in a multitude of environmental conditions.

6.2.2 Chemical sediment composition (MC experiment II)

The CH_4-C emissions [in ng] are six orders of magnitude smaller than the CO_2-C emissions [in mg]. Mostly CH_4 was absorbed in the MCs. A possible explanation could be due to aeration of the water overlying the sediment, which hindered CH_4 emission from the sediment by oxidising any escaping CH_4. It is interesting to note that only in the sand (S), silica sand (SS) and mixture of S and SS, noticeable amounts of methane were emitted and that these were the only treatments where any bacterial decay was detected. The CO_2-C emission did not

show any correlation with bacterial wood decay intensity and this reinforces the conclusion from the first experiment that CO_2 production cannot serve as an indicator for bacterial wood decay.

Although it was unintended, the sediment pH varied considerably between the treatments which had chemical additions. Nevertheless, the sediment pH seems to limit bacterial wood decay intensity when it is outside an "optimal" range, i.e. below pH 7 and above pH 8.5. However, this picture is obscured by the fact that some bacterial wood decay was detected at pH levels lower and higher than the proposed optimal range. As a general rule, bacterial activity is highly influenced by the pH of the growth medium and it is likely that bacterial wood decay intensity was affected by pH together with variations in nitrogen and phosphorus concentrations in the sediment. Conclusions related to the sediment pH are based on values measured at the end of the experiment and it is assumed that the final pH of the sediment was reached shortly after the experiment commenced.

The "optimal" pH range found in the experiment is supported by Boutelje and Göransson 1975) who measured ground water pH values of 7.2 - 8.0 surrounding bacterially decayed wooden foundation piles in Stockholm. Ground water in contact with archaeological wood and wooden foundation pilings decayed by bacteria showed a pH range of 7.0 to 8.5 with two values lower than 7.0 and one value higher than 8.5 (Huisman et al. 2007b; Kretschmar et al. 2007b).

There are indications that a negative relationship exists between bacterial wood decay intensity and sediment nitrogen concentration if it is presumed that sediment pH obscured, but did not totally govern bacterial wood decay intensity. This might indicate that in sediments with a very high C/N ratio or a generally very low carbon and nitrogen concentration, the high C/N ratio of wood is still "interesting" for the bacteria as a food source. Moreover, bacterially decayed wood was reported to have high N concentrations (Boutelje and Göransson 1975; Gelbrich et al. 2007) which might be related to wood with high nitrogen concentrations and a consequently lower C/N ratio providing an attractive substrate for bacterial wood decay. Following on from this idea, one might put forward the hypothesis that under nitrogen-poor or generally nutrient-poor conditions, wood is more susceptible to bacterial wood decay then under nutrient-rich conditions. Thus we favour the following conclusions as a basis for further research:

Wood surrounded by water saturated sediment with low nitrogen content is more likely to be affected by bacterial wood decay than wood in sediments with medium to high nitrogen contents. We draw this conclusion from the finding that nitrate addition to the sediment resulted in no bacterial decay in the wood samples. However, this might not in all cases be true as sediment samples with higher total N concentrations have been found surrounding quite intense bacterially decayed wooden pilings (Boutelje and Göransson 1975).

In addition, sulphate supplements to the sediment seemed to prevent bacterial decay in wooden samples and thus we concluded that sulphate addition might be a possible measure to inhibit bacterial wood attack. To consolidate the findings from MC experiment II the sulphate sediment addition was further tested in MC experiment III.

6.2.3 Prevention method (MC experiment III)

In order to achieve rapid results to confirm wood decay by bacteria, kapok fibres offer a real alternative because of their ease of manipulation and their lignocellulose composition. (Fengel and Przyklenk 1986; Khahili et al. 2000). Sediment glucose addition reduced bacterial kapok decay. Further sulphate addition totally inhibited bacterial decay with one exception. Within the time frame of the experiment of only four weeks glucose and sulphate addition was able to hinder initial bacterial kapok decay.

Glucose was added as an alternative food source for bacteria. The high microbial activity is very likely to reduce the redox potential considerably. Hence the effect of glucose addition is two fold: on the one hand glucose is an alternative food source for wood degrading bacteria and on the other hand its breakdown utilises oxygen acceptors which then are not available for the wood decaying bacteria. The second effect was enhanced even more by sulphate addition. Sulphate reduction took place and further reduced the redox potential.

7. Overall discussion

7.1. Which environmental characteristics favour bacterial wood decay?

This work was focused on wooden foundation pile surroundings. Pile foundations are normally in use when the bearing capacity of the sediment is too low to support buildings. This is mainly the case in sediments with peat layers, clay layers or unstable organic lagoon deposits as in Venice for instance. Therefore, only such sediments were investigated. However, sampling sites in Amsterdam were an exception because the ground level was raised by superimposing sea sand over the original peat sediment. Nevertheless, bacterial wood decay was found in all sampling sites but with different intensities.

A comparison of physico-chemical site conditions at a severely and a little bacterial decayed foundation site in Amsterdam (Chapter 5) studied seasonal changes in the sediment water composition. For the sampling at the foundation pile surroundings (Chapter 4) only one sampling campaign was conducted for the investigation of the foundation pile surroundings. The measurements, therefore, were only representative for the conditions at this time. Fiedler (2000) reported that redox potentials in soils were most influenced by ground water table fluctuations followed by temperature and precipitation. However, the sampled sites of this study were all water logged, therefore, influence of these factors is only relevant when foundation pile heads lay in the water table fluctuation range. At the severely degraded site (Chapter 5) fluctuations of ground water table and varying sediment water chemistry, especially sulphate, calcium and iron were observed over the measurement period of one year. Sediment water chemistry mainly conductivity, being representative for major ions, showed seasonal variations. However, no relation between major cation and wood decay was reported so far.

Nevertheless, when variation in environmental factors itself is regarded as factor it was noticed that at sites with more variation in the ground water table bacterial wood decay was pronounced (severely degraded site (Chapter 5) and air and water circulation treatment microcosm experiment I (Chapter 6)). The resulting variations in redox potential could provide micronutrients e.g. cobalt, manganese, copper, which are easier liberated under varying conditions than under static ones. Furthermore, it is speculated that under varying conditions bacterial populations are more active because they need to adapt to environmental

changes. These changes might inspire bacterial communities to explore wood as a new food source.

It is concluded that bacterial wood decay is present under nearly all environmental conditions but the velocity hence the decay intensity varies. There are presumably a multitude of bacteria creating the characteristic erosion bacteria decay which together have wide ecological amplitudes.

7.2. Is oxygen a perquisite for bacterial wood decay?

One of the most crucial questions for a better understanding of the process of bacterial wood degradation in water saturated sediments is, if and to which extent oxygen has to be available to stimulate bacterial growth that severe damage may occur. However, measurements of very low oxygen concentrations (<0.3 mg L^{-1}) require special precaution to prevent air oxygen intrusion, whereas in aerobic soil, platinum electrodes are not a reliable indicator of redox status (Esslington 2004). The combination from oxygen measurements in oxic conditions with redox potential measurements in suboxic and anoxic conditions provides additional information on a broad range of environmental conditions. The role of oxygen in bacterial wood decay is, therefore, discussed together with the redox potential measurements.

Bacterial wood decay occurred under anoxic condition in the microcosm experiment I, although it developed slower, compared to a treatment with oxygen supply. Around the majority of sampled wooden foundations with bacterial wood decay in Europe anoxic conditions prevailed. Monitoring of oxygen concentration and redox potential at two differently bacterial decayed wooden foundation sites in Amsterdam measured mostly redox potentials which preclude the presence of oxygen (anoxic range). Very low redox potentials, although not measured but deduced from the vigorous methane production, achieved by glucose and sulphate addition prevented bacterial kapok decay after 28 days in the microcosm experiment III. Intensive lab experiments with wood degrading bacteria indicated that an infection is impossible in open containers, but will spread out immediately if the same container is sealed from air contact after infection (Björdal and Nilsson 2005).

It is proposed that bacterial wood decay proceeds quickly in the suboxic (Eh 414 – 120 mV) redox potential range (manganese and iron reduction) but that it also occurs under anoxic

conditions proceeding slowly. This is in agreement with findings from Björndal (2000), who found a depth gradient in decay and related it to oxygen concentration differences, which can be conveyed to different redox zone present in different depths of river sediments.

Therefore we propose that:

- a suboxic redox potential range is optimal for bacterial wood decay, whereas decay exists under anoxic conditions but proceeds slower.
- bacterial wood decay exists without oxygen but traces are accelerating the decay.

7.3. Is eutrophication promoting bacterial wood decay?

In the presented investigations the main focus was the influence of nitrogen on bacterial wood decay. At the investigated foundation sites in sandy sediment the presence of higher nitrogen and organic carbon concentrations in the surroundings of the wood seems in general to slow down bacterial wood decay. This was seen in the monitoring of the two differently decayed foundations in Amsterdam (Chapter 5) and at three foundation pile sites with sandy sediments (Dordrecht, Zaandam, Amsterdam) at the sampling of the pile surrounding (Chapter 4). Sediment nitrogen addition in microcosms prevented bacterial decay for 155 days (Chapter 6, MC experiment II).

At the foundation sites in pure sand the presence of higher nitrogen and organic carbon concentrations in the surroundings of the wood seems in general to decrease bacterial wood decay. At wood collected from BACPOLES project sampling sites Gelbrich et al. (2007) measured higher nitrogen concentrations in bacterial decayed wood than in sound control wood. Furthermore, an investigation from Line (1997) indicates that improved nitrogen supply can considerably increase bacterial wood decay under waterlogged conditions. In this case a consortium of nitrogen fixating bacteria was identified as nitrogen supplier, which lived at wooden piping at the border of water saturated conditions. A study investigating aerobic fungal wood decay using different ^{15}N contents of materials could prove that organic nitrogen compounds were actively transported via fungal hyphens towards the wood. In wood the C/N ratio was thereby lowered and it became a better microbiologically accessible C-source (Filley et al. 2001). However, it might be possible, that wood degrading bacteria are also driven by different gradients of nitrogen availability and that this influences wood decay

patterns. This process known as chemo taxis is especially in nutrient poor environments of importance for the spatial distribution of microorganisms (Ginn et al. 2002).

In sandy sediments with low organic carbon content a bacterial community adapted to low nutrient conditions exists. They possibly show little activity due to limited carbon supply (although, bacteria exist in aquifers with total carbon concentrations as low as 2.9 mg L^{-1}, Barcelona 1984). Lignocellulose from wooden foundation piles is a complex carbon source which requires special enzymes for its brake down (Eaton and Hale 1993). Nevertheless, in environments with low nutrient concentration wood might provide a valuable C-source. Microbial communities adapted to low nutrient concentrations might be able to degrade wood.

However, it suggested that in other sediment types with better nutrient availability e.g. clay a more active bacterial community is able to co-metabolically degrade wood, which could explain earlier findings (Boutelje and Göransson 1975).

The theory that eutrophication is favouring bacterial wood decay is promoted by the considerations about fungal wood decay. Wood with lower C/N ratios is quicker broken down. In nitrogen rich surroundings wood will take up nitrogen by diffusion, although radial diffusion in wood is low (Holger Militz, personal communication). An alterative route of nitrogen rich ground water to the wood could be the vertical transport in wood driven by pressure differences between foundation pile head and tip. Vertical transport in pine was found to be higher compared to spruce (Klaassen 2007b) which correlates to observed susceptibility differences to bacterial wood decay between this two species. The nitrogen enriched wood is then better degradable by fungi as well as by bacteria.

7.4. Future work

Further investigations should select various sample sites in an area with comparable sediment type (i.e. clay, peat and sand) with similar building age and wood species of foundation poles and try to relate sediment and sediment water chemistry differences to differences in bacterial wood decay.

The role of nitrogen in bacterial wood decay should be investigated further by employing the ^{15}N technique to verify whether sediment nitrogen is taken up by bacteria during wood decay. More evidence is needed whether in sandy sediment bacterial wood decay proceeds faster if low nitrogen concentrations are present.

Further research is needed to separately identify pH effects and impacts of nutrient additions on bacterial wood decay.

The negative effect of methanogenic condition on bacterial wood decay might be an interesting field for further research.

8. Conclusions

8.1. Sampling of the foundation pile surrounding

Bacterial wood decay was found under very different sediment and sediment water conditions in wooden foundation piles.

The results indicate that there might be a relation between sediment water nitrogen concentration and the intensity of bacterial wood decay.

8.2. Investigating chemical sediment conditions at bacterial decayed wooden pile foundation sites in Amsterdam

Oxygen concentration, redox potential and ground water flow velocity can not explain decay intensity differences at two differently decayed wooden foundation pile sites with comparable sediment profiles. Seemingly the distance of the pile head to the average ground water level is the best explanation for the bacterial wood decay differences at the investigated sites. At the less bacterial decayed foundation sites significantly higher total sediment water nitrogen and organic carbon concentrations were found.

From the so far existing results it is concluded that the difference in nitrogen concentration between wood and its surrounding might be the controlling factor for bacterial wood decay and not the nitrogen concentration in the surrounding of the wood itself. Additionally, it seems that the seasonal variability of chemical and physical ground water constituents is a bacterial wood decay favouring factor.

8.3. Microcosm experiments

It can be concluded from the findings that bacterial wood decay can proceed without free oxygen present but that it is more intense if oxygen is available. However, only differences in the velocity of bacterial wood decay were observed, resulting in differences of decay intensity. Under the investigated conditions no differences in the general occurrence of bacterial wood decay were evident.

There are indications that in sandy sediment a negative relation between bacterial wood decay intensity and sediment nitrogen concentration exists if presuming that sediment pH obscured but not totally governed bacterial wood decay intensity. Following this idea might lead to the hypothesis that under nitrogen poor conditions wood is more susceptible to bacterial wood decay than under nutrient rich conditions.

Although, no strong evidence was presented there are hints that the sulphate addition to sediment might as least for 155 days inhibit bacterial wood decay. When glucose is added to the sulphate containing sediment nearly no bacterial decay in kapok was found after 28 days.

8.4. Overall findings

Taking all the evidence of bacterial wood decay into account following picture can be drawn:

Bacterial wood decay is present under nearly all environmental conditions but the velocity of decay varies. There are presumably a multitude of bacteria creating the characteristic erosion bacteria decay which together have wide ecological amplitudes.

The presence of oxygen traces accelerates bacterial wood decay but does not seem to be a perquisite for the occurrence.

It was proposed that under nitrogen poor conditions wood is more susceptible to bacterial wood decay than under nutrient rich conditions, although this is only true for sandy sediment. For other sediment types being frequently surrounded by wooden pile foundations i.e. clay and peat separate investigations are needed.

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10. Acknowledgement

I am grateful to Prof. Dr. Gerhard Gerold for accepting me officially as his student at the department of Landscape ecology of the Göttingen University and for taking over the referee. Furthermore, I like to thank Prof. Dr. Friedrich Beese who gave valuable ideas and acted as co-referee. I am very much in depth to Prof. Dr. Norbert Lamersdorf with whom working together was always inspiring. As my supervisor he succeeded in motivating me, corrected endless pages of writing and accepted my erratic working schedule.

I want to say thank you to Dr. Jan Ahlers, who as my boss at the Federal Environmental Agency gave me a downtime, which was crucial for the termination of the thesis.

I would like to give my special thanks to Thomas Nilsson and Charlotte Björdal from the Department of Wood Sciences of the Swedish Agricultural University Uppsala who shared a lot of their knowledge of bacterial wood decay with me. I want to say thank you to the partners in the BACPOLES Project for the good cooperation and discussion of the results especially to Hans Huismann and Rene Klaassen.

For organisation of the sampling sites and support during sampling I am in depth to: Herman Keijer, Christiaan van Stigt and all other field technicians from Fugro Ingenieursbureau BV, Amsterdam; Tamara Vernimmen from The National Service for Archaeological Heritage in Amersfoort, The Netherlands; Giovanni Abrami and from Ecopiano Engineering SRL, Padua, Italy; Mr. Kühl and all field technicians from the “Archäologisches Landesamt Schleswig-Holstein”.

I am thankful to Jana Gelbrich from the Institute of Wood Biology and Technology of the Göttingen University for the cooperation in the microcosm experiment and help with finding the right citation from her well organised literature collection.

I appreciate the commitment of Friederike Conrad who realised the practical work of the second microcosm experiment as her Masters thesis. I also want to express my gratitude to the team at the Institute of Soil Science and Forest Nutrition, at Göttingen University, which analysed sediment samples chemically, build redox probes and discussed findings with me. I am especially grateful to Norman Loftfield who made the gas measurements possible and to Heiner Flesser who contributed the deciding idea to the microcosm experiment. Furthermore,

I say thank you to the other PhD student the Institute of Soil Science and Forest nutrition for the good time at the institute.

For prove reading of the final manuscript and suggestions on structure I am in depth to: Sonja Paul, Ingo Schöning, Dirk Berthold, David Kuntze, and Gertraud Kretschmar.

Ganz herzlich möchte ich mich bei meiner Mutter bedanken, welche mir früh beigebracht hat nicht aufzugeben und mich immer unterstützt hat, ihr ist diese Arbeit gewidmet.

Finally I am in depth to the European Union for the financial support of this work as part of the EU-BACPOLES-Project under EVK4-CT-2001-00043.

11. Curriculum vitae - Lebenslauf

Name:	Kretschmar, Ev Iris
Geboren am/in:	18.08.1975 in Berlin Mitte
Familienstand:	ledig
Staatsangehörigkeit:	deutsch

Schulische Ausbildung

Sep. 1990 - Juni 1995	Friedrich-List-Oberschule (Gymnasium) in Berlin-Pankow
Sep. 1982 - Juni 1990	3. Oberschule "Georg Krausz" in Berlin-Pankow

Studienverlauf

Okt. 1995 - Dez. 2001	Studium des Technischen Umweltschutzes an der TU-Berlin
Juni 1998	Vordiplom im Technischen Umweltschutz
Okt. 1998 - Sept. 1999	Studium: Environmental Science and Ecology an der Lancaster University, Großbritannien
Sept. 1999	Abschluß: Masters (MSc) Environmental Science and Ecology
Juni 2000 - April 2001	Diplomarbeit an der Universidad de La Frontera, Temuco/ Chile
Dez. 2001	Abschluss als Dipl.-Ing'in. Technischer Umweltschutz

Praktika

Okt. - Nov. 2001	Fachpraktikum, Umweltbundesamt Fachgebiet II 5.4, Berlin
Sept. - Okt. 2001	Fachpraktikum, Protekum Umwelt GmbH, Oranienburg
Febr. - März 2001	Fachpraktikum, Paneles Arauco, Trupan/Chile
Juli - Sept. 1995	Technisches Grundpraktikum, Berliner Wasser Betriebe

Beruflicher Werdegang

Seit Juni 2006	Wissenschaftliche Mitarbeiterin im Umweltbundesamt im Fachgebiet IV 1.6, Prüfung des Umweltrisikos von Bioziden
März 2005 - Juni 2006	Wissenschaftliche Mitarbeiterin im Umweltbundesamt im Fachgebiet IV 2.4, Ökotoxikologische Bewertung von Stoffen
April 2002 - Jan. 2005	Wissenschaftliche Mitarbeiterin am Institut für Bodenkunde und Waldernährung der Universität Göttingen, Bearbeitung des EU-Projektes BACPOLES: „Preserving cultural heritage by preventing bacterial decay of wood in foundation poles and archaeological sites“