General Introduction

Groundwater is a major source for our drinking, industrial, and agricultural water needs worldwide. However, contamination of aquifers with organic and inorganic compounds threatens the long-term value and exploitation of groundwater resources. Detailed knowledge of factors that control the fate of groundwater contaminants is therefore of great importance. The strong influence of groundwater oxidation state on the fate of contaminants is well known. For example, chromium and uranium are soluble (mobile) under oxidizing conditions (Blowes, 2002; Senko *et al.*, 2002). In contrast, reducing conditions keep iron and manganese in solution by preventing the precipitation of their insoluble hydroxides at neutral pHs (Appelo and Postma, 1993). The fate of organic contaminants in groundwater is particularly dependent on the oxidation state of groundwater, since carbon occurs in a wide range of oxidation numbers (IV to -IV). For example, chlorinated solvents are more degradable under reducing conditions, while aromatic compounds (*e.g.* BTEX) are more degradable in oxic groundwaters (Bradley *et al.*, 1998; Nielsen *et al.*, 1995; Schreiber and Bahr, 1999; Skubal *et al.*, 2001). Aim of this thesis is to contribute to the knowledge of how reactive components in aquifer sediments affect the oxidation state of groundwater. The oxidation state of groundwater is controlled by thermodynamic imbalances that drive reduction-oxidation (redox) reactions during which electrons are transferred from a reductant (electron donor) to an oxidant (electron acceptor).

Chromate (CrO_4^-) and chlorinated hydrocarbons (*e.g.* TCE) are examples of contaminants with oxidizing properties (Fig. 1.1). Oxygen, nitrate and sulfate are the major oxidants in pristine groundwater. Besides these dissolved oxidants, solid iron and manganese oxides are important sediment-associated oxidants (Fig. 1.1). Reductants present in the aquifer consume these oxidants sequentially along a groundwater flow path in an order that mainly depends on their relative oxidation potential (Fig. 1.1). Consequently, dissolved oxygen initially present in shallow groundwater is removed at depth by naturally occurring biogeochemical processes, leading to aquifers that are free of oxygen (anoxic). Only under sufficiently depleted oxygen concentrations, the reductive transformation of nitrate (NO_3) to dinitrogen (N_2) gas occurs (Hiscock *et al.*, 1991; Korom, 1992; Tiedje, 1988). This process, known as denitrification, involves a multitude of intermediate electron transfer steps (Fig. 1.2). Commonly, denitrification in groundwater is coupled to the oxidation of sediment-associated reductants, such as pyrite (Böhlke and Denver, 1995; Kelly, 1997; Postma *et al.*, 1991) and organic matter (Bengtsson and Bergwall, 1995; Obenhuber and Lowrance, 1991; Smith *et al.*, 1991; Trudell *et al.*, 1986).

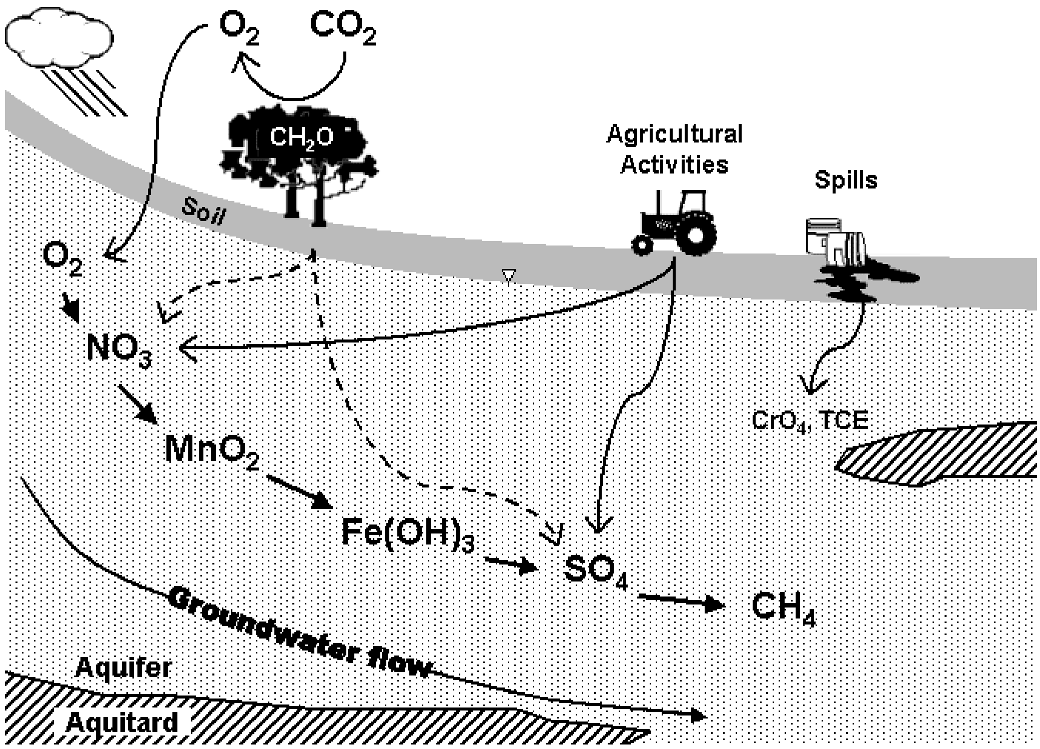

Figure 1.1 **Oxidant sources and sequence of reduction reactions in groundwater: aerobic respiration, NO_3-reduction, Mn-reduction, Fe-reduction, SO_4-reduction and CO_2-reduction (methanogenesis). Solid lines represent predominant sources. Dashed lines indicate additional sources.**

Redox processes are generally mediated by microbes that derive energy from the transfer of electrons. The amount of dissolved organic matter in most pristine groundwaters (<1 mg C/l) is too small and recalcitrant to create oxidant-depleted conditions (Aiken, 1985; Frimmel, 1998; Pettersson *et al.*, 1994; Thurman, 1985). Only when easily degradable organic compounds are excessively present (*e.g.* landfill leachate, petroleum spills), oxidant-limited conditions may occur. Otherwise, microbial metabolism is inherently limited by the availability of organic substrate or other potential reductants (Chapelle, 2000). Thus, while the *sequence* of oxidant consumption depends largely on their relative oxidative strength, the reactivity of reductants dominantly controls the *rate* of oxidant consumption. Therefore, to understand and predict the direction and magnitude of redox-related changes in the chemistry of both contaminated and pristine groundwater systems, detailed knowledge on the factors that control the reduction capacity of aquifers is essential.

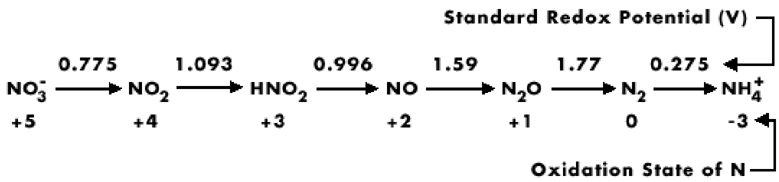

Figure 1.2 **The range in oxidation states of nitrogen. Denitrification involves the transfer of electrons during the reductive transformation of nitrate-N (V) to harmless dinitrogen (0) gas. Ammonium-N (-III) is the most reduced form of nitrogen and is the end product of dissimilatory nitrate reduction (Tiedje, 1988).**

1.1 REDUCTION CAPACITY OF AQUIFERS

The reduction capacity of aquifer sediments determines the extent to which natural attenuation of contaminating oxidants such as chromate or nitrate occurs (Fig. 1.1). In addition, it negatively affects the efficiency during the remediation of reducing contaminants (*e.g.* petroleum), since sedimentary reductants will compete for injected oxidants (Baker *et al.*, 2000; Barcelona and Holm, 1991; Broholm *et al.*, 2000; Heron and Christensen, 1995; Nelson *et al.*, 2001; Schäfer and Kinzelbach, 1996; Schreiber and Bahr, 1999).

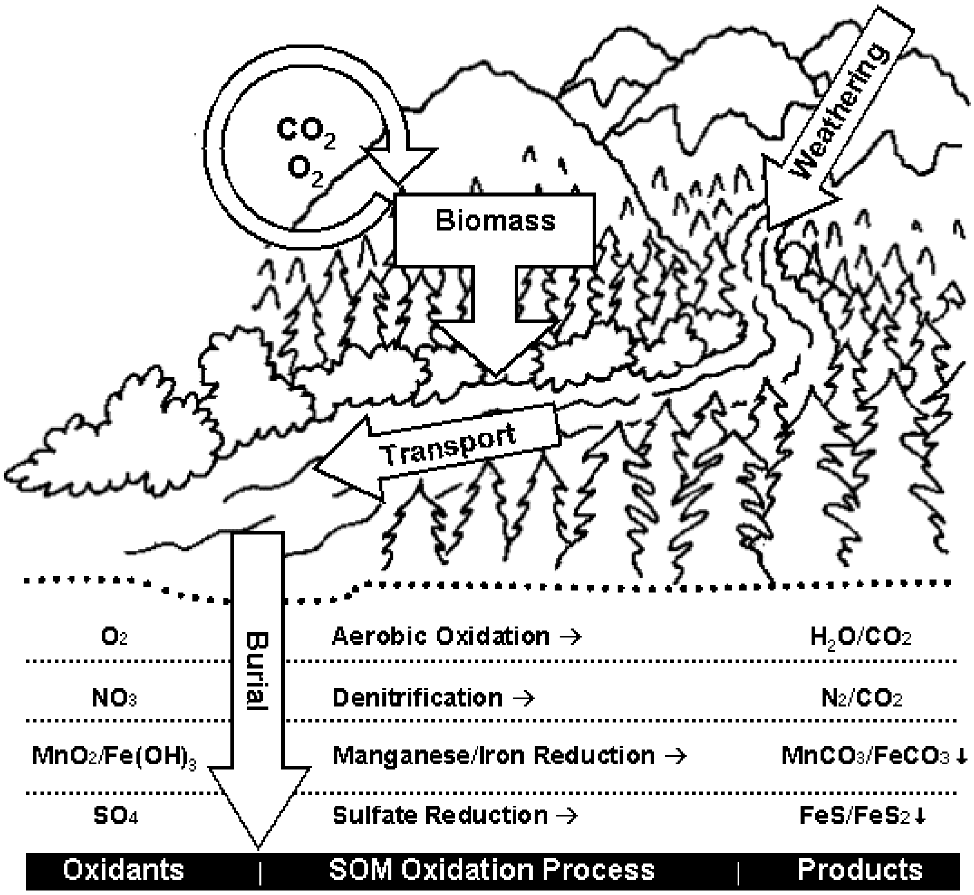

Figure 1.3 **The incorporation of sedimentary organic matter (SOM) during sediment deposition and subsequent diagenetic SOM oxidation processes. Aerobic oxidation and denitrification results in a loss of sediment reduction capacity. During manganese and iron reduction, the precipitation (↓) of mineral reductants retains sedimentary reduction capacity derived from SOM. Based on an illustration by Karen Hart.**

To understand the reduction capacity of aquifer sediments, knowledge of the amount, type and reactivity of sedimentary reductants present is crucial. Sedimentary organic matter (SOM) and a range of minerals that contain reduced sulfur, iron or manganese are potentially reactive in aquifers. For example, the anaerobic degradation of labile SOM during early sediment diagenesis components may drive the precipitation of pyrite (FeS_2), siderite ($FeCO_3$) or other mineral reductants (Berner, 1971). Therefore, the occurrence of these diagenetic processes affects the nature of the reduction capacity of aquifer sediments (Fig. 1.3). These secondary reductants are generated at the expense of labile SOM components (Berner, 1971; Sagemann *et al.*, 1999). The composition of SOM is thus a critical control in determining the nature of the reduction capacity of sedimentary aquifers, as it 1) influences the reactivity of SOM as a reductant and 2) controls the importance of mineral reductants that were formed during early diagenesis.

1.2 COMPOSITION OF SEDIMENTARY ORGANIC MATTER

The importance of SOM as a reductant in the redox chemistry of groundwater systems is long known (Freeze and Cherry, 1979; Johns, 1968; Plummer, 1977; Thornstenson and Fisher, 1979), but its molecular composition is still largely unexplored. Consequently, SOM in aquifers is generally referred to in ill-defined terms such as refractory, humic, amorphous or kerogen, without molecular verification of its nature. To date, research on the composition and degradation of organic matter has primarily focused on soils and marine surface sediments, environments that are significantly richer in organic matter than sandy aquifers (Fig. 1.4). As a result, numerous comprehensive books and thorough reviews on the nature of organic matter are available, mainly in the context of soil fertility, climate reconstruction and hydrocarbon source rock potential (*e.g.* Hedges and Oades, 1997; Stevenson, 1994; Tissot and Welte, 1984; Tyson, 1995).

The predominant source of SOM is the burial of primary biomass with accumulating sediment (Tyson, 1995). Plant and microbial biomass consist of complex organic mixtures and the relative abundances of organic compounds vary with biomass type (Kogel-Knabner, 2002). Therefore, the compositional variation of SOM reflects to some extent differences in the composition of the biomass source. Marine phytoplankton is a considerable source for amino acids and short-chain lipids (Camacho-Ibar *et al.*, 2003; Grossi *et al.*, 2001; Sun *et al.*, 2002), while land plants are predominantly composed of the carbohydrate-based macromolecules. In addition, higher plants contain lignin compounds that provide strength to support tree trunks and branches and comprise 5−30 % of dry biomass. These heterogeneous polyphenolic macromolecules are specific for higher land plants and thus act as biomarkers for a terrigenous SOM origin (Hedges and Oades, 1997; Tyson, 1995).

Although the initial composition of SOM strongly reflects the composition of the biomass source, oxidation reactions alter the composition of SOM during and after burial (Fig. 1.3). Most of buried SOM (63−98%) does not survive beyond early diagenesis (Tyson, 1995). In particular, the mineralization of labile compounds such as plankton-derived amino acids is faster than of macromolecular compounds such as lignin (Cowie and Hedges, 1992; Cowie *et al.*, 1992; Henrichs, 1993; Tegelaar *et al.*, 1995). Consequently, SOM degradation rates in soils and marine sediments range in orders of magnitude, depending on the reactivity of the compounds present (Henrichs, 1993; Kogel-Knabner, 2002).

The mineralization rate of organic matter partly depends on oxidant type. Studies have indicated that the rates for aerobic and anaerobic degradation of labile organic compounds are similar (Henrichs and Reeburgh, 1987; Lee, 1992). However, recalcitrant organic components such as lignin or macromolecular aliphatics degrade much faster under aerobic than under anaerobic conditions (Canfield, 1994; Hulthe *et al.*, 1998; Kristensen and Holmer, 2001). The chief explanation for these observations is that during aerobic degradation, oxygen not only functions as an oxidant, it also serves as a co-substrate for enzymes (oxygenases) that aid the oxidation of recalcitrant aromatic and aliphatic compounds. As a result of the lack of these oxygenases, anaerobic degradation proceeds through less efficient pathways, such as benzoyl-CoA metabolism (Harwood *et al.*, 1999).

For an assessment of the overall potential reactivity of SOM, its bulk composition must be characterized. While several analytical techniques are available (Kögel-Knabner, 2000), common elemental analysis is not sufficiently specific to cover the wide range of organic compounds present. In addition, the abundance of macromolecular compounds in biomass (Kogel-Knabner, 2002) makes SOM unavailable to any direct analytical approach (Saiz-Jimenez, 1994). ^{13}C NMR spectroscopy and other spectroscopic techniques are now widely used for the chemical characterization of SOM (Kögel-Knabner, 2000). These techniques provide information about the nature of carbon environments such as functional groups or aromaticity, and the non-destructiveness and the lack of major pretreatment requirements are big advantages for samples. However, the low organic matter contents and the presence of Fe-bearing paramagnetic compounds limit their applicability of SOM in aquifer sediments. Furthermore, these techniques do not provide information on the molecular associations of SOM. Pyrolysis is a powerfull thermal degradation technique that allows the characterization of the building blocks of complex macromolecular organic matter when coupled to gas chromatograph and mass spectrometer (Py-GC/MS). It is frequently used to characterize the bulk composition of organic matter in both soils and sediments (Chiavari *et al.*, 1994; Kögel-Knabner, 2000; Levy, 1966; Saiz-Jimenez, 1994; Saiz-Jimenez and De Leeuw, 1986). Although several pitfalls exist, it is currently the main technique available for the molecular bulk characterization of complex SOM (Chiavari *et al.*, 1994).

1.3 REACTIVITY OF SOM IN GROUNDWATER SYSTEMS

Rates of SOM oxidation in aquifer sediments are several orders of magnitude lower than observed in environments that recurrently receive fresh organic matter, such as marine surface sediments (Chapelle and Lovley, 1990; Jakobsen and Postma, 1994). In groundwater systems with an ample, continuous supply of fresh labile organic matter (*e.g.* land-fill leachate), the availability of oxidants commonly limits organic matter degradation rates (Chapelle, 2000). In addition, environmental conditions, such as nutrient level, temperature or acidity potentially control microbial activity (Atlas and Bartha, 1998).

A number of studies have shown that not the addition of nitrate but the addition of a labile carbon source, such as glucose, significantly increased denitrification rates in groundwater systems (Bengtsson and Bergwall, 1995; Bradley *et al.*, 1992; Hill *et al.*, 2000; Obenhuber and Lowrance, 1991; Smith and Duff, 1988; Starr and Gillham, 1993). This indicates that neither microbial activity nor the amount of oxidants is rate limiting and supports the general idea that the availability of SOM controls the rate of its degradation in aquifer sediments.

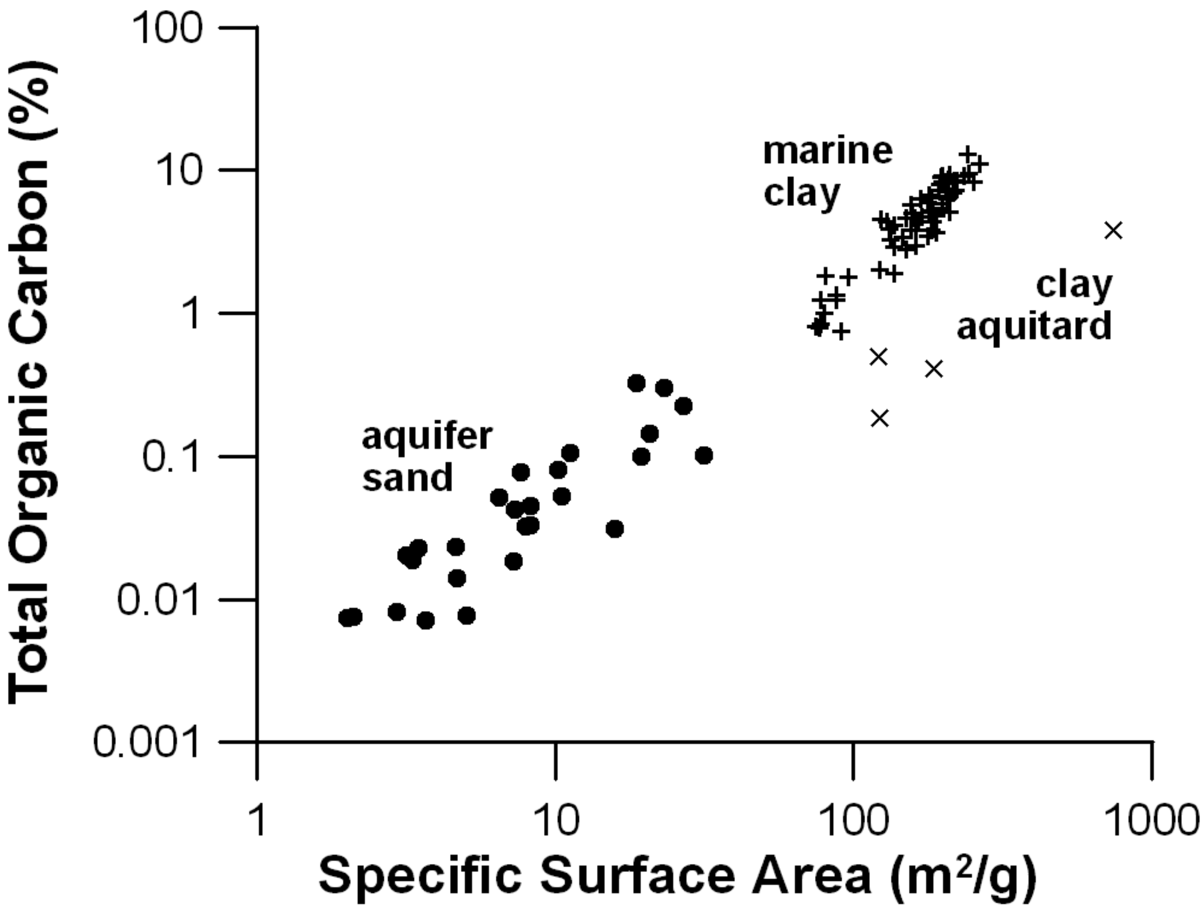

Figure 1.4 **Cross plot of total organic carbon versus specific surface area of the mineral phase. A preliminary study (unpublished results) at the 't Klooster site (Fig. 1.5) provided the data for the aquifer sands. Data for marine clay is taken from a study on black shale (Kennedy *et al.,* 2002). Clay aquitard data are taken from a study on four different aquitards (Allen-King *et al.,* 1995). All specific surface areas (SSA) were determined by sorption of ethyl-glycol monoethyl (Churchman *et al.,* 1991).**

Both its accessibility (physical) and degradability (chemical) potentially control the availability of SOM in aquifer sediments. Physical limitations on its reactivity occur at a grain scale when particle–organic compound interactions protects a part of the organic matter against microbial degradation. Studies have indicated a relationship between SOM availability and sorption to mineral surfaces in both marine clay sediments (Keil *et al.*, 1994; Mayer, 1994a; Mayer, 1994b; Mayer, 1999) and soils (Chorover and Amistadi, 2001; Salmon *et al.*, 2000; Sollins *et al.*, 1996). In groundwater systems, it has been shown that microbes in clay aquitards are unable to mineralize the SOM present due to pore size restrictions (Chapelle and Bradley, 1996; Chapelle and Lovley, 1990; McMahon and Chapelle, 1991). In a preliminary study, a positive relationship was found between the specific surface area and total organic carbon contents of aquifer sands (Fig. 1.4, unpublished results). While considerable scatter in the data exists, the general trend compares favorably with data for clayey sediments (Allen-King *et al.*, 1995; Kennedy *et al.*, 2002). Therefore, the interaction of SOM with mineral surfaces may decrease its availability in aquifer sediments.

Alternatively, SOM may be chemically refractory towards oxidation. From studies on organic matter in soils and marine sediments, it is generally recognized that its reactivity decreases with continuing degradation. More precisely, the most labile compounds are consumed at a higher rate, resulting in an overall decrease of SOM reactivity with time. Built on this notion, several descriptive models have incorporated SOM fractions with different reactivities to account for the decreasing reactivity of SOM with time (Berner, 1980; Middelburg, 1989). However, these fractions are arbitrary and no tools exist to assess the size and reactivity of these different kinetic pools (Almendros and Dorado, 1999; Gleixner *et al.*, 2002).

1.4 SCOPE OF THIS STUDY

This thesis focuses on the role of SOM as a reductant in aquifer sediments. Using pyrolysis-GC/MS, the molecular composition of SOM is characterized and the controls on its reactivity are assessed.

As stated earlier, SOM generally co-occurs and is frequently even closely associated with other sedimentary reductants in aquifer sediments. Therefore, the relative contribution of SOM to oxidant consumption during sediment oxidation depends on the reactivity of other reductants present. The amounts of these reductants present depend on the diagenetic history and provenance of the sediment. For example, pyrite and Fe(II)-bearing glauconite are commonly formed in marine depositional environments, while siderite is predominantly formed in terrestrial settings (Berner, 1971; Postma, 1982). While the reactivity of SOM in aquifers is either chemically or physically controlled, the oxidation of these reductants under pH-neutral conditions is mainly determined by surface oxidation kinetics. Therefore, the precipitation of metal hydroxide on mineral surfaces is an impediment that controls their reactivity (Nicholson *et al.*, 1990; Postma, 1983; Postma, 1990). The co-occurrence of several potentially reactive sedimentary reductants in aquifer sediments complicates the isolated study of SOM reactivity upon exposure to oxidants. Therefore, the separation of and the controls on the contributions of various reductants to the reduction capacity of aquifer sediments is another aim of this study.

Aquifer sediments from two drinking water production sites were studied (Fig. 1.5). The Langerak site is located in the central part of the Netherlands. Here, a confined sedimentary aquifer is recharged with water from the River Lek. Proposed future induced riverbank infiltration will increase the oxidant loadings of NO_3 and O_2. The site ‘t Klooster is located in the eastern part of the Netherlands. Here, knowledge on the reactivity of aquifer sediments is particularly important as the excessive use of agricultural fertilizers on sandy soils cause elevated nitrate concentrations in shallow groundwater (Fraters *et al.*, 1998; Hefting and de Klein, 1998; Pomper, 1989; Reijnders *et al.*, 1998; van Beek *et al.*, 1994; van Beek and Vogelaar, 1998).

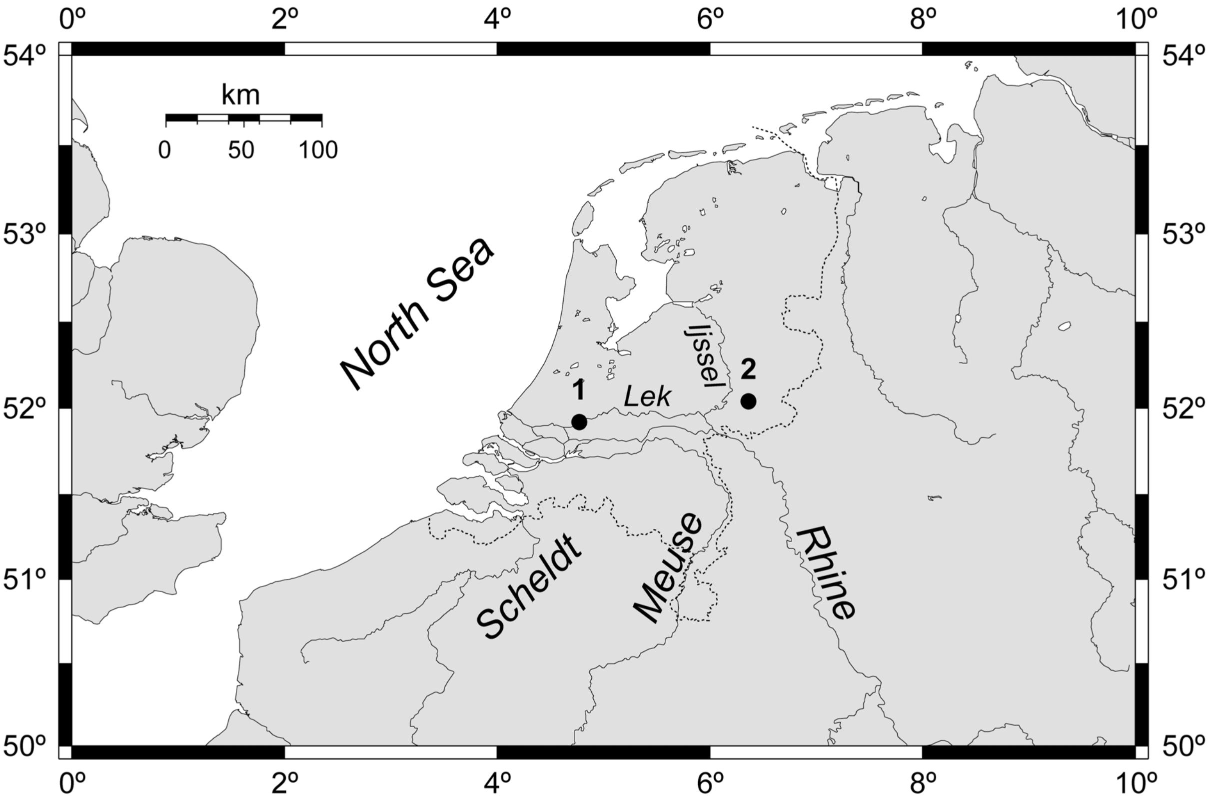

Figure 1.5 **Location of the Langerak (1) and ‘t Klooster (2) aquifers in the Rhine–Meuse delta. The Langerak site is located along the River Lek. The ‘t Klooster site is located in between the River Rhine and River Ijssel. Dotted line represents the Dutch national boundary.**

1.5 Outline of this Thesis

This chapter serves as an introduction for the following research chapters. *Chapter 2* describes the design and development of a fluidized-bed reactor for anaerobic biogeochemical sediment incubations; the developed fluidized-bed reactor was tested during denitrification experiments described in *Chapter 4*. In *Chapter 3*, sediments from the Langerak aquifer were characterized for the presence and reactivity of potential reductants. The reactivity towards oxygen was determined during sediment incubations. A method is developed to discriminate between contributions from SOM, pyrite and siderite oxidation based on CO_2/O_2 ratios and sulfate production. This method is also applied for the sediment incubations describe in *Chapters 5 and 6*. In *Chapter 4,* the nitrate reduction potential of anaerobic sediments from the Langerak aquifer is assessed using fluidized-bed (*Chapter 2*) and batch reactor experiments. The geochemical and microbial controls on denitrification are discussed.

Chapter 5 describes the molecular composition of SOM in aquifer sediments selected from a marine and fluvio-glacial formation at the Klooster site. Molecular indications on the degradation status of SOM are linked with the reactivity of SOM as observed during aerobic incubation experiments. *Chapter 6* discusses the molecular composition of SOM in different geological formations at the Klooster site. The controls on SOM preservation as well as the presence of pyrite and ferroan carbonates in aquifer sediments at this site are assessed. The controls on the reduction capacity and on the contributions of various reductants are discussed using aerobic sediment oxidation experiments. Lastly, *Chapter 7* provides a synthesis of the thesis, in which the main findings are summarized and discussed, and where implications and future research directions are considered.

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