Sulfur Cycling in Swedish Arable Soils

A Chemical Perspective

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Cover: Ryegrass growing in soil from Fjärdingslöv (FYM-treatment) with (+S) and without (-S) mineral sulfur fertilization. Fors field experimental site in the background.
(photo: Kristin Boye, edited by Shawn Boye)

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Abstract

Sulfur (S) is an essential plant nutrient. Decreased S deposition in combination with a switch to high-analysis N/P-fertilizers has increased the need for S fertilization. Thus, soil research directed at understanding soil sulfur properties and processes has intensified. However, the methodology at hand has been insufficient for determining relationships between soil properties, S cycling and S availability to crops.

In this thesis, recently developed methods were used to study the effect on soil S by two management systems, livestock production and arable crop production, at five different locations within a Swedish long-term fertility field experimental series. In an open incubation study, and a pot trial, where isotopic labeling (^{35}S) was used to trace S transformations, S cycling rates were higher in the livestock system, especially in one soil (Orup). The S delivering capacity of all soils was too low to avoid S deficiency in ryegrass without mineral S application. Observed differences in S cycling patterns could not be satisfactorily explained by soil properties; however, multivariate analyses indicated net S mineralization was negatively related to C/N-ratios and SO_4^{2-} content. The extent of organic S stabilization through organomineral association and physical protection within microaggregates was investigated by an extraction/dispersion method. The relative distribution between the pools varied between soils, with the residual (non-extractable) pool always being largest; however, only the physically protected fraction was negatively related to plant S uptake. All soil organic S pools were involved in S transformations, although the residual pool was less active than the other pools. Chemical speciation of S in soils and soil fractions was determined by S K-edge X-Ray Absorption Near-Edge Structure (XANES) spectroscopy. A new method for fitting spectra provided reliable quantification of S species by using internally calibrated spectra of dilute (30mM) model compounds. The response of S speciation to management system differed between soils, but highly oxidized S dominated in the organomineral fractions, and intermediate forms of oxidized S in the residual fraction.

In conclusion, soil organic S speciation can be accurately quantified by S K-edge XANES spectroscopy. The speciation differs between organomineral associated S and residual S. Treatment effects are dependent on soil type, but S cycling is stimulated by long-term farmyard manure application, as seen in the livestock system.

Keywords: soil sulfur, plant availability, long-term effect, farmyard manure, organic matter, organomineral stabilization, S K-edge XANES, quantitative sulfur speciation

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Dedication

For Aidan, who taught me that every moment holds a seed of happiness.

Rather than seeing sulfur as an element of fire and brimstone, I've always seen it as an element of life, odors and all.

Rusty Tanton

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Boye, K., Nilsson, S.I., Eriksen, J. (2009). Net sulfur mineralization potential in Swedish arable soils in relation to long-term treatment history and soil properties. *Biology and Fertility of Soils* 45 (7), 743-751.

II Boye, K., Eriksen, J., Nilsson, S.I., Mattson, L. (2010). Sulfur flow in a soil-plant system – effects of long-term treatment history and soil properties. *Plant and Soil* 334 (1-2), 323-334.

III Almkvist, G., Boye, K., Persson, I. (2010). *K*-edge XANES analysis of sulfur compounds: an investigation of the relative intensities using internal calibration. *Journal of Synchrotron Radiation* 17, 683-688.

IV Boye, K., Almkvist, G., Nilsson, S.I., Eriksen, J., Persson, I. (2011). Quantification of chemical S species in bulk soil and organic S fractions by S K-edge XANES spectroscopy. *European Journal of Soil Science*, accepted for publication. DOI: 10.1111/j.1365-2389.2011.01391.x.

The contribution of Kristin Boye to the papers included in this thesis was as follows:

I Planned the experimental work together with the co-authors. Performed the practical laboratory work, apart from S analyses. Performed data analyses and writing, with assistance from the co-authors.

II Planned the experimental work together with the co-authors. Performed the practical work, with some assistance. Performed data analyses and writing, with assistance from the co-authors.

III Participated in the planning and experimental work. Assisted in data analyses and writing.

IV Participated in the planning and experimental work. Performed data analysis and writing, with assistance from the co-authors.

Abbreviations

ANOVA	Analysis of variance
CR	Crop residues
DMSO	Dimethyl sulfoxide
FYM	Farmyard manure
LCF	Linear combination fitting
MW	Molecular weight
OrgS-D	Organic sulfur extracted by acetylacetone from dispersed soil
OrgS-ND	Organic sulfur extracted by acetylacetone from non-dispersed soil
OrgS-Res	Residual organic sulfur
PLS	Partial least squares regression
Sol-S	Inorganic and organic sulfur soluble in potassium phosphate
XANES	X-ray absorption near edge structure

1 Introduction

Sulfur (S), an essential element for all living organisms, is required for the synthesis of several important biochemical substances, such as the amino acids methionine, cysteine and cystine, the vitamin biotin, and co-enzyme A. Nonetheless, for a long time sulfur was mainly associated with negative phenomena, such as acid rain, toxic substances and to some extent problems associated with farming on acid sulfate soils. The latter is a serious problem in some parts of South-East Asia and on the Australian continent (Dent, 1986). The importance of S as a plant nutrient was largely overlooked in soil science research, mainly because the incidental supply of S via deposition and S-containing nitrogen (N) and phosphorus (P) fertilizers was usually sufficient to match crop demand. However, since concerns about acid rain were raised in the 1980s, sulfur emissions from fossil fuel burning has decreased remarkably in western Europe and North America (Figure 1), as smoke stacks

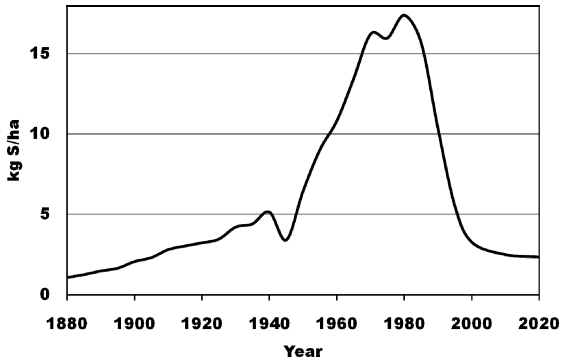


Figure 1. The dramatic change in sulfur deposition in Western Europe and North America during the past 140 years, exemplified by annual S deposition in central Sweden. Data from EMEP, recalculated according to Schöpp et al. (2003). Figure kindly provided by Jens Fölster, Department of Water and Environment, SLU, Uppsala, Sweden.

became equipped with flue gas desulfurization and steps were taken to change to less S-containing substances. During the same period and within agriculture in the Western world, the older types of N/P-fertilizers, which contained up to 24% sulfur (in ammonium sulfate), were largely replaced by high analysis fertilizers containing at most a few percent of S.

The reduced input of S combined with intensified farming and larger S removal in harvests depleted the S stocks in soils and in the 1990s, sulfur deficiencies began to be reported (Ceccotti, 1996). Sulfur deficiency can cause marked yield loss and reduce the quality of crops and forage (Haneklaus et al., 1992; Wang et al., 2002; 2008; Zhao et al., 2006a). In addition, S deficiency decreases resistance to pathogens (Dubuis et al., 2005; Falk et al., 2007; Walters and Bingham, 2007) and reduces nitrogen utilization efficiency (Ahmad and Abdin, 2000; McGrath and Zhao, 1996; Schnug and Haneklaus, 1993). As S is a relatively abundant and cheap element, fertilization with S has now become standard over large areas. However, knowledge of how to optimize the amount and timing of fertilization is still insufficient and there is evidence of negative impacts of non-optimized S fertilization. For example, sulfur fertilization can decrease selenium (Se) content in crops, which is problematic for animal and human nutrition in Se-poor soils (Shinmachi et al., 2010; Stroud et al, 2010). Sulfur can also induce eutrophication in freshwater wetlands, as the precipitation of iron sulfide releases iron-bonded phosphates (Smolders and Roelofs, 1993; Lamers et al., 2002).

In organic farming systems, regulations restrict the use of mineral fertilizers and stipulate animal feed should be organically grown, without any artificial additives. Consequently, in these systems it is more difficult to avoid S deficiency in crops and animals. As interest in organically produced food increased simultaneously with the emergence of S deficiency problems, there was a mounted focus on understanding the mechanisms of S supply from soil and organic S sources to plants.

2 Aim

The overall aim of this thesis was to study the long-term influence of farming management systems on soil S cycling patterns in different soils and to explain observed patterns in S behavior, primarily through investigating the chemical properties and dynamics of the soil organic S pool. This work included five soils from a long-term soil fertility field experimental series, where each soil hosts two different management systems: livestock production and arable crop production. The S cycling patterns were studied in a laboratory incubation experiment and an outdoor pot trial, where carrier-free $^{35}SO_4^{2-}$ was used to trace the S processes.

The specific objectives were:

- To assess the long-term effect of the management systems on net S mineralization and plant availability of S in different soils (Papers I and II).
- To assess the effect of mineral S application on S cycling processes and crop growth (Paper II).
- To determine relationships between observed S cycling patterns, basic soil properties and degree of physiochemical protection of soil organic S from microbial processes (Papers I and II).
- To develop a reliable method for quantifying organic S species in soil samples by X-ray Absorption Near Edge Structure (XANES) spectroscopy (Papers III and IV).
- To determine the relative distribution of S species in bulk soil and in organomineral fractions, as affected by long-term management system and soil type (Paper IV).

3 Background

3.1 The Soil Sulfur Cycle

Plants need sulfur in amounts comparable to those of phosphorus and are almost exclusively dependent on the uptake of inorganic sulfate (SO_4^{2-}) from the soil substrate for their S supply. However, in general, more than 95% of S in soils is in organic form, which means plants are dependent on mineralization, the transformation of organic S to inorganic SO_4^{2-}, to satisfy their sulfur requirement. There are two main pathways for mineralization, biochemical and biological (McGill and Cole, 1981), and both are mediated by microorganisms. Thus, S supply to plants is dependent on the activity and composition of the microbial communities in the soil (Kertesz and Mirleau, 2004), which are affected by soil temperature, moisture regime, plant-root interactions (Maynard et al., 1985; Castellano and Dick, 1991), cropping system (Eriksen et al., 1995a; Vong et al., 2003; 2004) and, organic matter quantity, quality[1] and availability (Mirleau et al., 2005).

3.1.1 Biochemical Mineralization

Biochemical mineralization is the hydrolysis of sulfate esters ($R\text{-}O\text{-}SO_3$) catalyzed by sulfatase enzymes (Fitzgerald and Strickland, 1987). These enzymes are normally external to the microorganisms, although intracellular sulfatases are important in e.g. *Pseudomonas* species (Kertesz and Mirleau, 2004). Sulfatase activity appears to be negatively correlated with inorganic SO_4^{2-} levels (Maynard et al., 1985; Prietzel, 2001; Saviozzi et al., 2006) and as biochemical mineralization of sulfate esters requires an active energy

1. Organic matter quality is an ambiguous term, which is often used without specific definition. Here it relates to the composition and degradability of organic substances and the relative abundance of important nutrients, i.e. the nutritional value for microbes.

investment from microorganisms, this process is considered as being controlled by the microbial need for S (Eriksen, 2009). Due to the higher liability for hydrolysis, sulfate esters are generally considered a transitory group of organic S (McGill and Cole, 1981; McLaren et al., 1985); however, there are indications that sulfate esters are less labile than previously considered as they appear to accumulate during decomposition (Schroth et al., 2007; Solomon et al., 2005; 2009).

3.1.2 Biological Mineralization

In contrast to biochemical mineralization, which directly provides the microorganisms with S, biological mineralization is considered a byproduct of microorganisms decomposing organic matter in search of carbon (C) for satisfying their energy demand (McGill and Cole, 1981). Some of the S ingested in this process will be used by the microorganisms for cell synthesis and only excess S is excreted as sulfate (Freney and Stevenson, 1966). As a consequence, S mineralized in this way should be dependent on the C/S ratio of the substrate material and have a close correlation with carbon and nitrogen (N) mineralization (Kowalenko and Lowe, 1975; Gharmakher et al., 2009; Tabatabai and Al-Khafaji, 1980).

3.1.3 Immobilization

Concurrent with mineralization of S is an opposite process, immobilization, whereby inorganic SO_4^{2-} is incorporated into organic compounds by microorganisms. This process is stimulated by readily available C compounds (Maynard et al., 1985; Ghani et al., 1992; 1993a; Eriksen, 1997a; 1997b; Knights et al., 2001; Vong et al., 2003) and nitrogen (Vong et al., 2003) and is dependent on both the C/N and the C/S ratios of the organic matter. The immobilization rate also correlates with sulfatase activity (Vong et al., 2003) and sulfate esters may constitute the first step of both immobilization (Ghani et al., 1993a; Saggar et al., 1981) and mineralization (Norman et al, 2002).

3.1.4 Organomineral Stabilization

Organic matter can be adsorbed onto clay surfaces via bridging of polyvalent metal ions. The resulting organomineral complexes play an important role in soil aggregation (Tisdall and Oades, 1982), but this stabilizing effect means organic matter is protected from microbial breakdown, especially if it is contained inside microaggregates, where it is assumed physically inaccessible for microorganisms (Ladd et al., 1993). Thus, a considerable amount of the organic S pool is probably passive (Eriksen et al., 1998). The chemical

properties and dissolution rates of the protected S pool, and its effect on S cycling processes, are still largely unknown.

3.1.5 Net S Mineralization

The availability of S to crops is dependent on several simultaneous processes (Figure 2) that are reliant on different factors: this means the assessment of the net result, i.e. net S mineralization (or immobilization), is complicated. There have been many attempts to determine correlations between various soil properties and net S mineralization in order to provide an easy way of estimating the rate of S supply to crops from organic sources and to make reliable fertilizer recommendations. However, to date, the only general rule this work has generated, is that substrate C/S ratios above 400 result in net S immobilization and ratios below 200 result in net S mineralization (Barrow, 1960; Reddy et al., 2002; Tabatabai and Chae, 1991).

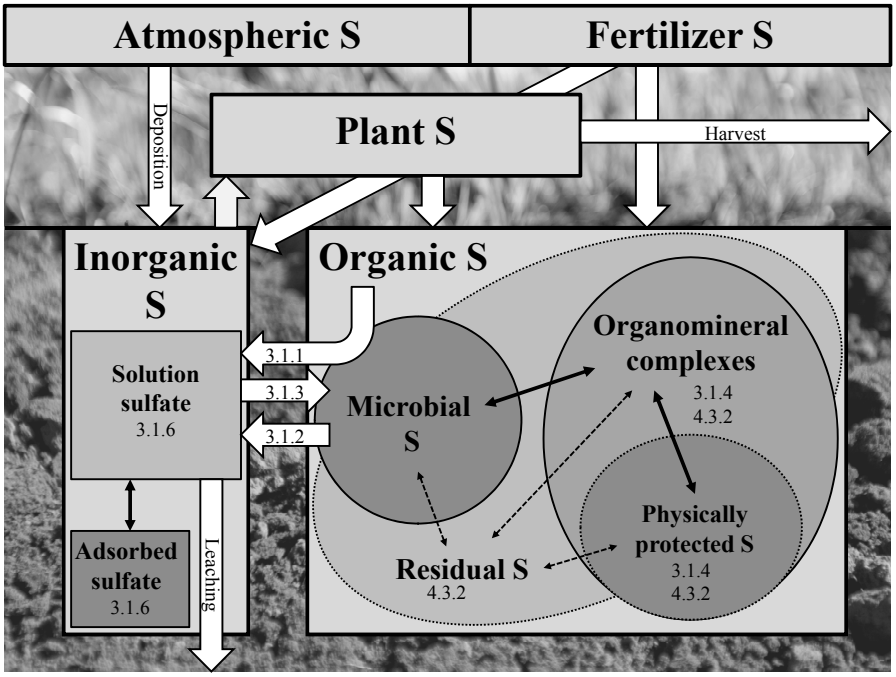


Figure 2. Conceptual model of the soil sulfur cycle in Swedish arable soils. The numbers refer to the sections in the text where the processes and fractions are described. Dashed arrows depict processes of uncertain magnitude.

3.1.6 Fertilizers

In order to provide reliable fertilizer recommendations, it is insufficient to estimate the rate of sulfate release from the organic pools in the soil. The fate of fertilizer S has to be known as well; and this is dependent on the type of fertilizer applied, S cycling and leaching properties of the particular soil, and the properties of the crop being grown.

Mineral fertilizers generally contain S in the form of inorganic sulfate or elemental S^0. Inorganic sulfate is directly available for plant uptake, but is prone to rapid leaching in most arable soils. Sulfate can be adsorbed onto mineral surfaces and temporarily stored in the soil. However, the adsorption is pH-dependent and in soils with a pH>6, sulfate adsorption capacity is negligible (Curtin and Syers, 1990). Elemental S^0 has to be transformed into sulfate by microorganisms before it can be utilized by the plants; thus, elemental S^0 is recommended on e.g. sandy soils prone to leaching. However, oxidization of S^0 causes a decrease in pH and can have a negative impact on the microbial composition and activity (Gupta et al., 1988). Regardless of the chemical speciation of the mineral S that is applied, there appears to be very limited build-up of S stocks from mineral fertilizers even long-term (Eriksen and Mortensen, 1999; Knights et al., 2001).

The soil organic S pool can be replenished with long-term supply of farmyard manure (FYM) or other organic fertilizers (Eriksen and Mortensen, 1999; Knights et al., 2001; Reddy et al., 2001; Singh et al., 2007). However, the rate of sulfate release from organic fertilizers is dependent on the same processes as S mineralization from organic S pools in the soil, and is just as difficult to estimate. FYM appears to stimulate net S mineralization in the long-term (Knights et al., 2001; Reddy et al., 2001), but it is unclear why and to what extent this stimulation occurs. There is no indication organic S from FYM is more readily mineralized short-term than bulk soil organic S (Eriksen, 2009), which emphasizes the importance of long-term experiments for evaluating different management systems.

3.2 Methodological Background

Predicting the S supply to crops requires an understanding of the mechanisms and processes of the soil S cycle. This entails identification of soil properties that affect S transformations and accurate quantification of the identified properties and of the processes they control.

3.2.1 Methods for Quantifying Soil S Mineralization

Incubation experiments are commonly used to estimate the rate of net S mineralization in soils. However, the correlation of the results with plant uptake of S appears arbitrary (Maynard et al.,1985; Nzigueba et al., 2005; 2006; Pamidi et al., 2001), which renders the experiments unreliable predictors of plant S availability. Even so, if correctly planned, it is a relatively quick and convenient method for determining the potential net S mineralization and testing the influence of different factors on S cycling rates on a laboratory scale.

Pot and field experiments yield reliable information about the amounts of available S and net S mineralization in a field situation (Eriksen et al., 1995a; Vong et al., 2007). However, these methods are slow and costly and still provide only limited information about the mechanisms behind the observations, unless combined with isotopic labeling. The radioactive isotope ^{35}S is advantageous for tracing S originating from the soil inorganic sulfate pool or fertilizer S (Eriksen, 1997a; 1997b; Nzigueba, 2006; Vong et al., 2004), but recycling of S between different pools within the soil obscures the overall patterns (Eriksen, 2005). Work that is more specific is therefore needed to reveal the actual pathways.

Another limiting factor for understanding how soil properties affect S cycling patterns is that treatment effects, especially long-term effects, on soil S transformation rates are rarely studied on different soil types simultaneously. This undermines the possibility of making general conclusions and limits the value of the information gained in the experiments.

3.2.2 Methods for Determining Soil S Properties

Much focus in agricultural soil S research is put on studying the relationship between chemical properties of soil organic S and S transformations in soils. However, when the interest in soil S research began to flourish, it became evident the existing methods for determining soil S properties provided insufficient information for explaining the mechanisms and processes behind observed S cycling patterns. The search for improved methods is still an ongoing process.

HI/Raney-Ni Reduction Method

For many years, knowledge of the chemical properties of soil organic S was based on a complicated fractionation procedure in which hydroiodic acid (HI) reduces organic S (Tabatabai, 1982). HI only reduces sulfur not directly bound to carbon, resulting in a fractionation between HI-reducible S and C-bonded S (Table 1). This is generally interpreted as a division between sulfate esters and

Table 1. *Soil organic S fractions by the HI-reduction method (Tabatabai, 1982). Examples of functional groups within each fraction according to Freney (1986)*

HI-reducible	C-bonded (not reduced by HI)	
	Raney-Ni reducible	Not reduced by Raney-Ni
Sulfate esters, R-O-SO_3	Disulfides (e.g. cystine), R-S-S-R´	Sulfones, R-SO_2-R´
Sulfamic acids, R-NH-SO_3H	Thiols (e.g. cysteine), R-SH	Aliphatic sulfonic acids,
S-sulfocysteine, Cys-SO_3H	Thioethers (e.g. methionine), R-S-R´	Aliph-SO_3H
	Sulfoxides, R-SO-R´	
	Sulfinic acids, R-SOOH	
	Aromatic sulfonic acids, Aryl-SO_3H	

C-bonded S, as sulfate esters dominate the HI-reducible group. The C-bonded S can be further divided into Raney-Ni reducible S and S that is not reduced by this method. The Raney-Ni reducible S group contains reduced organic S and some forms of oxidized organic S. The fraction not reduced by Raney-Ni is considered to comprise unreactive oxidized C-bonded S (Zhao et al., 1996).

The amount of sulfate ester S in a soil is potentially important for the mineralization rates, as sulfate esters are susceptible to mineralization external to microbes and thus, possibly more labile than C-bonded S. Even so, the information gained by HI/Raney-Ni fractionation is limited and the explanatory value of the different fractions in terms of S cycling rates and plant-availability of S is low (Ghani et al., 1993b; Eriksen et al., 1995a; Eriksen et al., 1998; Knights et al., 2001; Mansfeldt and Blume, 2002). The problem being that the method is indirect and destructive and the resulting groups are operationally defined; each group contains several functional S-groups with varying chemical properties and stability.

X-ray Spectroscopy

In the late 1990s and the beginning of the 2000s, soil S researchers began to explore the possibility of S K-edge X-ray Absorption Near Edge Structure (XANES) spectroscopy for determining S speciation in soils (Prietzel et al., 2003; Solomon et al., 2003; Vairavamurthy et al., 1997; Xia et al., 1998). XANES is a non-destructive, direct method that provides the opportunity for determining all oxidation states of the sulfur present in soil and for identifying different chemical species (functional groups) of S. The principle behind XANES is that synchrotron generated X-ray radiation is absorbed by the S atoms: electrons are transmitted to unoccupied or partially occupied higher energy states (1s→3p) or are ejected into the continuum as photoelectrons.

This absorption edge occurs at slightly different energy levels depending on the electronic configuration (or oxidation state[2]) of the absorbing atom and on the geometry of neighboring atoms. Thus, the peaks in an S K-edge XANES spectrum provide specific information regarding which sulfur containing functional groups are present in the sample (Vairavamurthy et al., 1997). The species are commonly identified through deconvolution of the spectrum into a number of Gaussian peaks (theoretically corresponding to the 1s→3p transmissions) and arctangent step functions (theoretically corresponding to the ejection of photoelectrons), and then assigning each Gaussian peak to a group of S species (Solomon et al., 2009; Xia et al., 1998). There are several problematic assumptions involved in this procedure. One of the more obvious problems is that all S peaks are assumed to have a Gaussian shape, which is not true (*cf.* Figure 3). This can be avoided by applying another commonly used method: curve-fitting of actual spectra from model S compounds (Beauchemin et al., 2002; Vairavamurthy et al., 1997) by least-squares linear combination fitting (LCF).

S K-edge XANES spectroscopy has become popular in soil S research and has successfully been used to determine S speciation in extracts of humic and fulvic acids (Morra et al., 1997; Xia et al. 1998), and in particle size separates (Solomon et al. 2001), where S concentration is higher than in the original bulk soil samples. As the technique developed, enhancement of the S concentration became unnecessary and it is now possible to measure untreated bulk soil samples (Prietzel et al., 2007), which greatly improves the reliability of the results. The method is now generally accepted for qualitative determination of soil S species and has been used to demonstrate changes in S chemistry both during changes in land use (Solomon et al., 2003; 2005; Zhao et al. 2006b) and during organic matter decomposition (Schroth et al., 2007). The non-destructive, direct nature of the method, in combination with a more detailed determination and functionally based grouping of S species, provides the potential for explaining observed S cycling patterns.

However, problems still exist, with the most obvious being the need for a synchrotron facility to perform the analyses: this limits applicability in everyday research. In addition, it is difficult to distinguish between S species with similar oxidation states, e.g. inorganic sulfate/sulfate esters, or thiols/sulfides (Prietzel et al., 2003; 2007; Waldo et al., 1991). Another issue, which will be addressed in this thesis, is that in order to use XANES data in

2. The formal oxidation states of sulfur can range between –II and +VI, but are ambiguously assigned. Thus, it is more accurate to speak of electronic configuration or oxidation indices (Vairavamurthy, 1998).

statistical tests and correlation analyses, the relative abundance of different S species has to be determined; however, reliable quantification of S species is more difficult than qualitative analysis for several reasons, including:

1. Self-absorption of the signal within the sample can be a problem in high-concentration samples. Self-absorption alters the shape of the peaks making them broader with reduced amplitude (Figure 3). This can cause overestimation of the contribution from certain species, when high-concentration model spectra are used to fit low-concentration sample spectra.
2. The peaks change depending on factors such as pH (Pickering et al., 1998), complex formation with metal ions (Jalilehvand, 2006) and, whether the compound occurs in solution or in solid state (Pickering et al., 1998). Thus, the model compounds need to be in the same chemical and physical state as the corresponding compounds in the sample, which can be difficult to achieve.
3. In a sample with more than one S-containing compound, a reduced S compound of the same concentration as an oxidized S compound will have a smaller peak area. This is the result of a stepwise increase in absorption intensity as an electron is ejected into the continuum when the irradiation energy increases above the binding energy of the core electron (i.e. post-edge). The magnitude of the change in peak area is often assumed proportional to the number of ejected electrons (i.e. the number of 3p vacancies) (Waldo et al., 1991). However, the assumption that the step size is independent of the structural composition surrounding the S atom introduces uncertainty into the fit (≤ 15% according to Waldo et al., 1991), which can cause notable errors in the estimation of relative abundance.

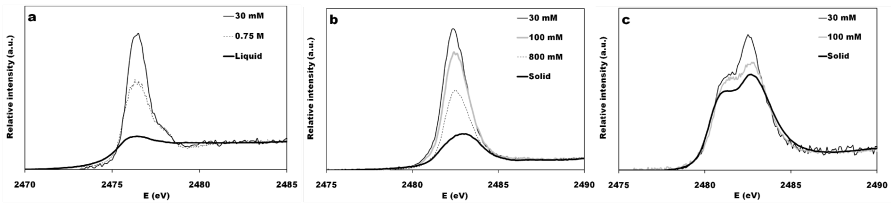


Figure 3. Absorption spectra for a) DMSO ($(CH_3)_2SO$, liquid and in aqueous solutions) b) sodium sulfate ($Na_2SO_4 \cdot 10H_2O$, solid and in aqueous solutions) and c) organic sulfate (Chondroitin sulfate ester, $C_{14}H_{19}NO_{14}SNa_2$, sodium salt and in aqueous solution).

Organomineral stabilization is important, and can explain the refractory behavior of a large part of the organic S pool (Eriksen et al., 1995b; 1995c; Eriksen, 1997a). This notion led to the development and use of several techniques for studying organic matter based on its susceptibility to microbial processes. These techniques include particle size separation (Hinds and Lowe, 1980; Anderson et al., 1981; Eriksen, 1996) and molecular weight (MW) fractionations (Keer et al., 1990; Eriksen et al., 1995c). Another technique is to use acetylacetone to break the organomineral bonds and disperse the microaggregates by ultrasound treatment; thereby releasing physically protected S (Keer et al., 1990; Eriksen et al., 1995b; 1995c; Eriksen, 1997a). The methods have provided evidence that a majority of the organic S in soils is passive due to physical protection within the microaggregates (Eriksen et al., 1995b; 1995c; Eriksen, 1997a) or association with clay mineral surfaces (Hinds and Lowe, 1980; Anderson et al., 1981; Eriksen, 1996) and/or high MW compounds (Keer et al., 1990; Eriksen et al., 1995c). Moreover, sulfate esters, as determined by HI-reduction, appear to be overrepresented in fractions associated with clay and high MW compounds (Keer et al., 1990; Eriksen, 1996); this could explain the low contribution of sulfate esters to S mineralization (Ghani et al., 1992; Zhou et al., 2005), despite them presumably being labile compounds.

The combined determination of chemical properties (MW and HI-reducibility) and physiochemical protection of soil organic S provides valuable information, but does not satisfactorily explain differences in S cycling rates between soils. One of the main issues is the low resolution of organic S speciation provided by the destructive, indirect, operationally based determination. Thus, the possibilities to reveal relationships between the processes and properties of soil organic S would improve, if the functional groups of chemical S species in organomineral associated fractions could be more accurately identified and quantified, e.g. by S K-edge XANES spectroscopy.

4 Materials and Methods

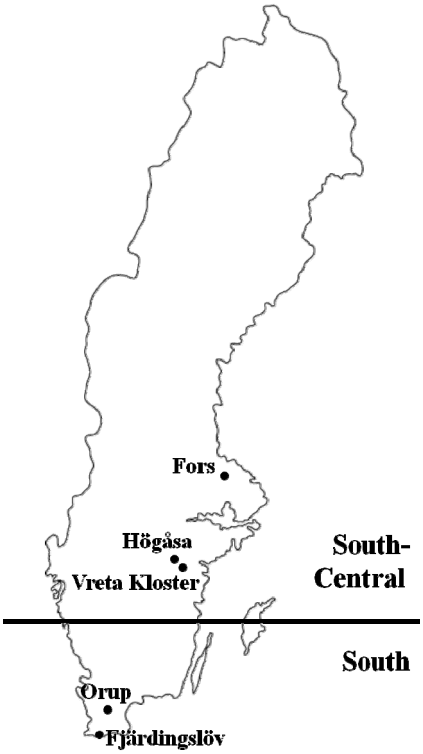
4.1 Long-term Field Treatments and Soils

The Swedish long-term soil fertility field experimental series (Carlgren and Mattsson, 2001) provides a unique possibility for studying long-term management and fertilization responses among different soil types. The experimental series was initiated between 1957 and 1966 on ten locations in south and central Sweden. There are two main treatments, a livestock production system and an arable crop production system, with two field replicates at each site. The only differences between the two systems are that in the livestock production system, ley replaces oilseed rape in the crop rotation, FYM is applied to the field, and crop residues (CR) are removed. Thus, the organic matter input quality differs between the systems. Within each management system there are several sub-treatments with varying application levels of mineral N, P and K fertilizer. Sulfur is not included in the fertility study, and mineral S has been applied to all fields across all treatments since 1998 to avoid S deficiency in the crops. As the aim of this project was to study the effect of the management systems on soil S cycling patterns, only the main treatments were relevant. However, when the field experiments were initiated, the older types of mineral NPK fertilizers were used, and these contained large amounts of mineral S (e.g. 13% in mono-superphosphate). In order to isolate the effect of management system on organic S quality, it was considered necessary to minimize the historic input of inorganic S. Thus, only the sub-treatment with no PK and normal N fertilization (to avoid problems due to N deficiency in the microbial community) was sampled in each main treatment. The specific details of the management systems are presented in Table 2 and the systems are henceforth referred to by their organic matter source, i.e. FYM (livestock production) and CR (arable crop production).

Table 2. *Fertilization and crop rotation in the two field treatments selected for this thesis (Carlgren and Mattsson, 2001). The differences between the South and South-Central sites are due to adaptations to differences in climate (Figure 4)*

	FYM		CR	
	South	South-Central	South	South-Central
Mineral N (kg N per ha per year)	100	82	100	82
Mineral S as $MgSO_4$[a] (kg S per ha per rotation)	84	84	84	84
Farmyard manure[b] (t fresh weight per ha per rotation)	20	30	0	0
Crop residues	Removed	Removed	Incorporated	Incorporated
Crop rotation	4 years	6 years	4 years	6 years
	Barley	Barley	Barley	Barley
	Ley 1 (1 cut)	Ley 1 (2 cuts)	Oil seed	Oats
	Winter wheat	Ley 2 (1 cut)	Winter wheat	Oil seed
	Sugar beet	Winter wheat	Sugar beet	Winter wheat
		Oats		Oats
		Winter wheat		Winter wheat

[a]Applied in spring the year after barley, since 1998.
[b]Applied in autumn after winter wheat in Southern sites and after ley 2 in South-Central sites.



Five locations with different soil types were selected from the ten sites that compose the field experimental series (Table 3, Figure 4). All five soils were included in the incubation experiment (Paper I), four were selected for the pot trial (Paper II) and three for the chemical speciation study (Paper IV). Before each experiment, soil samples (10 per plot) were taken from the plough layer (0-20 cm), and the sub-samples from the two field replicates were combined into one composite sample for each treatment at each site. More detailed descriptions on sampling and storing procedures are given in Papers I and II.

Figure 4. Location of experimental sites. The management practices differ slightly between South and South-Central sites due to climatic adaptations (Table 2).

Table 3. *Experimental sites and soil types*

Site	Latitude	Texture	Sand (%)	Silt (%)	Clay (%)	Classification	Included in papers
Fjärdingslöv	54°24' N	Sandy Loam	55.1	25.9	19.1	Oxyaquic Hapludoll[a] Haplic Phaeozem[b]	I, II
Fors	60°20' N	Silt Loam	28.3	54.9	16.9	Udic Haploboroll[a] Calcaric Phaeozem[b]	I, II, IV
Högåsa	58°30' N	Sandy Loam	75.4	17.3	7.4	Humic Dystrocryept[c] Arenic Umbrisol[d]	I, II, IV
Orup	55°49' N	Sandy Loam	60.2	29.0	10.8	Aquic Haploboroll[a] Haplic Phaeozem[b]	I, II, IV
Vreta Kloster	58°29' N	Silty Clay	8.1	49.1	42.9	Oxyaquic Haplocryoll[c] Haplic Phaeozem[d]	I

[a] Soil Taxonomy (Carlgren and Mattson, 2001)
[b] World Reference Base (Carlgren and Mattson, 2001)
[c] Soil Taxonomy (Kirchmann et al., 2005)
[d] World Reference Base (Kirchmann et al., 2005)

4.2 Experiments

4.2.1 Incubation Experiment (Paper I)

An open incubation technique was selected for determining net S mineralization potential and its relation to soil properties. In open incubation, the sulfate produced by mineralization is continuously leached and collected for analysis. The leaching is supposed to simulate plant removal of sulfate (Maynard et al., 1983; Tabatabai and Al-Khafaji, 1980; Valeur and Nilsson, 1993) and provides the possibility to follow the net S mineralization over time.

In preparation for the incubation study, the fresh soil samples were sieved (≤ 2 mm) and mixed with glass-beads (Ø 2 mm) at a 3:2 ratio, before being placed in drained Plexiglas tubes (3 replicates) (Figure 5). After two weeks of pre-incubation (18^{o}C, soil moisture at -10 kPa), the initial sulfate content was removed by leaching with 0.016 M KH_2PO_4. Thereafter, artificial sulfate-free "rain water"[3] was used to remove excess phosphate and regenerate a realistic salt concentration in the soil. The soils were incubated for 95 days in the same conditions as the pre-incubation and leached every two weeks with the artificial "rain water" to remove any SO_4^{2-} that had formed. At the end of the experiment, the soils were leached with 0.016 M KH_2PO_4 to ensure all SO_4^{2-} that had formed during the incubation period was removed.

All leachates were filtered (Millipore 0.45 μm filter) and analyzed for sulfate content by anion chromatography.

Figure 5. Experimental set-up of incubation experiment (left) and pot trial (right).

3. Ion concentrations in μmol per liter: K^+: 5, Na^+: 60, Ca^{2+}: 15, Mg^{2+}:10, NH_4^+: 60, NO_3^-: 30 and Cl^-: 145.

4.2.2 Pot Experiment (Paper II)

The incubation experiment was followed-up by a pot trial to measure actual S delivery from soil to plants. To simulate as natural climatic conditions as possible, the pot experiment was conducted outdoors in a restricted area with full-height netted walls and a glass-roof (Figure 5); to avoid wet deposition of S and access by animals and unauthorized people. The inorganic sulfate pool in the soil was labeled with isotopic sulfur (^{35}S) to enable better tracing of gross mechanisms. Additionally, the selection of ryegrass as the experimental crop, allowed for two harvests and, thus, further improved the possibilities of monitoring S mineralization (as measured by plant S uptake).

Four soils were selected for the pot trial. Vreta Kloster, with its high clay content, was excluded to avoid technical problems, such as clogging and difficulty separating roots from soil. Fresh soil samples, equivalent to 7 kg dry soil each, were used in the pots and the pots were kept at field capacity. The original inorganic sulfate pool was labeled with isotopic sulfate (carrier-free $H_2{}^{35}SO_4$) for determining the sources of plant S and flow between S pools within the soil. After two weeks of soil pre-incubation, Italian ryegrass (*Lolium multiflorum* cv. SW Fredrik) was sown and fertilized with a basal dressing of N, K, P, Mo, Mg, Mn and Cu to ensure no deficiencies apart from S deficiency would occur. Two experimental treatments were applied: no S fertilization (-S) and "optimized" mineral S fertilization (+S) (15 mg S per kg dry soil); with six replicates for each soil within both FYM and CR. In total there were 96 pots: 4 soils x 2 experimental treatments x 2 management systems x 6 replicates.

The grass was harvested twice during the experiment. At the first harvest (41 days after sowing), only shoots (≥ 6 cm above soil surface) were removed. At the end of the experiment (73 days after sowing), shoots, stubble (0-6 cm above the soil surface), and roots (from three of the six replicates) were harvested. Soil samples for post-trial analyses were collected from the three replicates where roots were not harvested. Thus, there were six replicates for above-ground plant variables and three replicates for soil, root and total variables.

4.2.3 XANES Experiments (Papers III and IV)

The S speciation in bulk soil from three sites (Fors, Högåsa and Orup) and extracts of organomineral associated S from two sites (Fors and Högåsa) was determined by S K-edge XANES. The selection of soils was made to maximize the differences in total S and distribution of S between organic fractions.

In order to obtain reliable quantification of S species in the samples, the problems associated with self-absorption and compound-dependent post-edge intensities needed to be minimized (see section 3.2.2). Therefore, a new

method was developed for data treatment of spectra from natural samples with a low S concentration. The fitting of sample spectra was by model spectra rather than Gaussian curves, the model compounds were diluted to avoid self-absorption, and the peak areas were internally calibrated by combining model compounds of the same concentration to ensure that post-edge intensities were accurate.

4.3 Analytical Methods

4.3.1 Basic Soil Properties

Basic soil properties were determined at the beginning of each experiment with standard methods described in Papers I and II. The averages between the two sampling occasions are presented in Table 4. In the pot experiment, total S was determined again after the experiment (data not shown), to allow for mass balance calculations.

Table 4. *Basic soil properties, averages between the two sampling occasions (Papers I and II)*

Soil	Field treatment	pH[a]	SO_4^{2-} [b]	Total S[c]	Total C[d]	Total N[d]	Anaerobic N mineralization[e]
			(mg kg^{-1} dry soil)	(mg kg^{-1} dry soil)	(g kg^{-1} dry soil)	(g kg^{-1} dry soil)	(mg day^{-1} kg^{-1} dry soil)
Fjärdingslöv	FYM	6.91	1.4	244	15.7	1.80	1.4
	CR	6.93	1.7	209	12.7	1.55	1.1
Fors	FYM	7.66	2.0	490	22.9	2.11	1.0
	CR	7.79	1.6	468	20.2	1.74	0.4
Högåsa	FYM	6.12	2.2	250	20.6	1.81	2.3
	CR	6.43	2.3	239	19.6	1.73	2.0
Orup	FYM	5.44	2.0	280	22.6	2.11	1.2
	CR	5.86	1.8	281	19.9	2.07	1.1
Vreta Kloster[f]	FYM	6.88	2.2	351	20.4	2.40	1.4
	CR	6.87	1.7	320	18.2	2.27	0.7

[a] in H_2O
[b] H_2O-extraction, ion chromatography
[c] $HNO_3/HClO_4$ digestion, ICP-AES
[d] dry combustion in CHN analyzer
[e] indication of microbiological activity (Drinkwater et al. 1996), only measured in Paper I
[f] soil only included in Paper I

4.3.2 Physically Protected S

The degree of organomineral stabilization and physical protection of S within microaggregates was determined on fresh soil by ultrasonication and extraction in acetylacetone, according to a method originally proposed by Keer et al. (1990), and later modified by Eriksen et al. (1995a; 1995b). The determinations were done before the incubation experiment and both before and after the pot experiment. Soil samples were extracted sequentially in three steps (Figure 6) and each step was repeated once before proceeding to the next step. This resulted in three fractions of decreasing degree of availability to microbes and one residual, inextractable fraction (OrgS-Res). The steps of extraction and resulting fractions were:

1. 0.016 M KH_2PO_4 – Inorganic and easily dissolved organic S (Sol-S)
2. 0.2 M acetylacetone – Physically unprotected organic S in organomineral complexes (organic S extracted from non-dispersed soil, OrgS-ND)
3. Ultrasonication (1800 J ml^{-1}) in 0.2 M acetylacetone – Physically protected organic S in organomineral complexes (organic S extracted from dispersed soil, OrgS-D)

All extracts were analyzed for total-S by ICP-AES, but the acetylacetone extracts were digested by HNO_3 prior to analysis, as acetylacetone extinguishes the flame of the ICP-apparatus. The S speciation in extracts from the Högåsa and Fors soils was determined by S K-edge XANES spectroscopy, as previously described.

4.3.3 Plant Analyses

All plant samples were dried, weighed, and ground. Total S was determined in all plant parts by ICP-AES after digestion with concentrated HNO_3. Total N was analyzed in shoots (dry combustion in a CHN analyzer) to ensure N deficiency had not occurred.

4.3.4 Radio-Isotope Analyses

The post-trial samples from the pot experiment analyzed for total S (i.e. plant parts, bulk soil and extracts from the physically protected S fractionation) were also analyzed for ^{35}S activity by liquid scintillation counting.

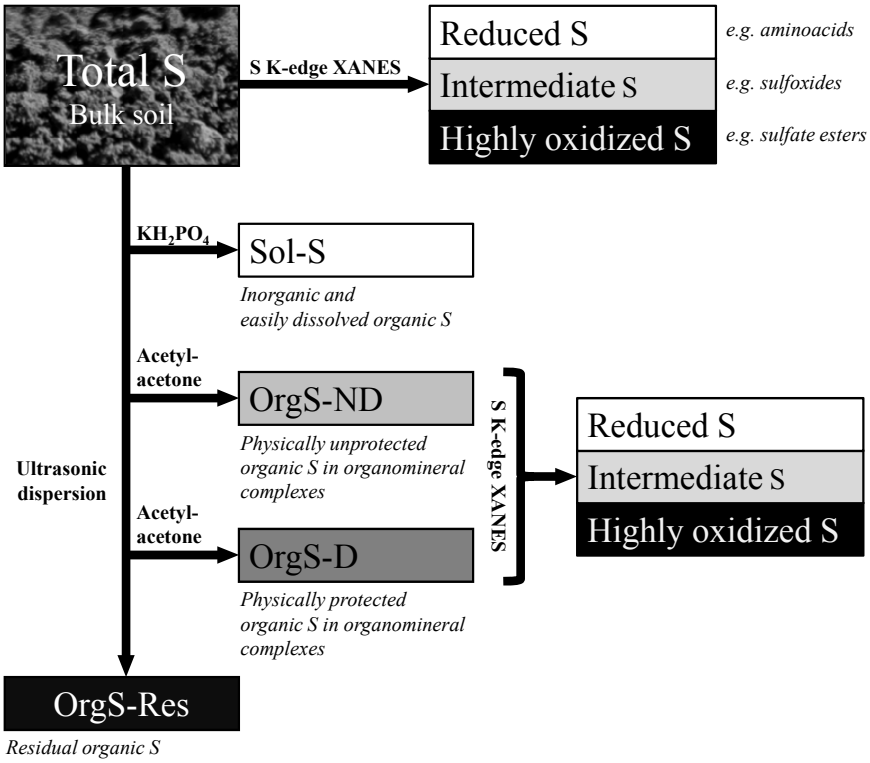


Figure 6. Overview of the fractionation of soil S according to organomineral association and physical protection and the determination of organic S speciation in bulk soil and organomineral fractions.

4.3.5 S K-edge XANES Spectroscopy

The S K-edge XANES Spectroscopy was performed at beamline I811 at the MAX-laboratory, Lund University, Sweden. All spectra were recorded in fluorescence mode, using a Lytle detector, and with a helium atmosphere at a slight over-pressure to minimize noise related to the absorption and scattering by air. The energy was calibrated by recording the S K-edge spectrum of solid sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) (Aldrich) immediately before or after every sample, and assigning the maximum of the first peak in the spectrum to 2472.02 eV (Williams et al., 1997). The raw XANES spectra were background-subtracted by a linear function extrapolated from the pre-edge region, and, if necessary, the same procedure was performed after the edge region to achieve a slope of zero in the post-edge region (E>2500 eV).

Model Compounds

Reference solutions (30 mM) for a number of model compounds (Table 5) were prepared by combining each model compound with dimethyl sulfoxide (DMSO), sodium sulfate or thiosalicylic acid. Using dilute solutions minimized the problems with self-absorption and 30 mM was found to be an optimal concentration for this purpose (Figure 3, section 3.2.2). The combination of two compounds at equal concentrations and well separated absorption peaks enabled internal calibration of post-edge intensities and relative maximum intensities. Internally calibrated model spectra were obtained by normalizing the peak intensity of DMSO at 2476.4 eV to 1.00 and linear regression fitting the individual spectra for the two combined compounds to the reference solution spectrum (Figure 7). DMSO was used as the primary internal calibration reference compound because it is chemically stable, soluble in both polar and non-polar solvents, and its absorption edge is clearly separated from typical reduced and oxidized S forms. Sodium sulfate and thiosalicylic acid, with edge peaks 2482.4 and 2473.4 eV respectively, were calibrated against DMSO and then used as internal calibration reference compounds when the absorption edge of a model compound coincided close to DMSO. In this manner, the maximum K-edge intensities of all model compounds were related to the normalized DMSO peak (Table 5, Figure 7).

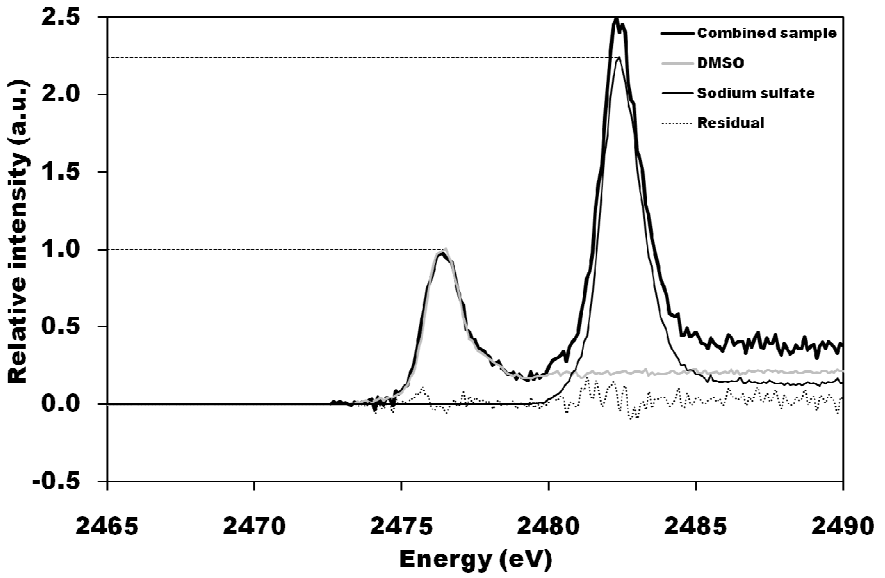


Figure 7. Normalized S K-edge XANES spectrum of an aqueous solution of DMSO (30mM) and sodium sulfate (30mM), with separation of the substances according to best fit with linear regression. Residuals from the linear regression are indicated by the dashed line.

Table 5. *Description of the internally calibrated model compounds included in the least squares LCF regressions. Compounds that contributed less than 2% to the preliminary fit were excluded in the final fit, as indicated in the last column*

	Function	Sulfur compound in solution	Calibration compound and solvent	Maximum peak (eV)	Additional peaks (eV)	Maximum Edge Intensity Relative DMSO	Line format in fitted sample spectra
Reduced S	Disulfide	L-Cystine, $[HOOCCH(NH_2)CH_2S]_2$	Sulfate, aq, buffer pH 7.0	2472.6	2474.1	0.59	
	Disulfide	Diphenyl disulfide, $(H_5C_6S)_2$	DMSO, *p*-xylene	2472.7	2474.4	0.53	
	Disulfide	Dibenzyl disulfide, $(H_7C_7S)_2$	DMSO, *p*-xylene	2472.8		0.74	not used
	Thiol	L-Cysteine, $HSCH_2CH(NH_2)COOH$	Sulfate, aq, buffer pH 7.0	2473.4		0.59	
	Thiol	Tiosalicylic acid, $HS(C_6H_4)COOH$	Sulfate, aq, buffer pH 7.0	2473.5		0.50	
	Thio ether	L-Methionine, $HOOCCH(NH_2)CH_2CH_2SCH_3$	Sulfate, aq	2473.6		0.82	
Inter-mediate S	Sulfoxide	Diphenyl sulfoxide, $(C_6H_5)_2SO$	Thiosalicylic acid, *p*-xylene	2476.0	2477.6	0.57	
	Sulfoxide	Dimethyl sulfoxide, $(CH_3)_2O$	Aq	2476.4		**1.00**	
	Sulfite	Dimethyl sulfite, $(CH_3O)_2SO$	Sulfate, aq	2477.7		1.1	
	Sulfite	Sodium sulfite, Na_2SO_3	Sulfate, aq	2478.3		1.1	
Highly oxidized S	Sulfone	Tetramethylene sulfone, $C_4H_8O_2S$	Sulfate, aq	2479.6		1.1	
	Sulfone	L-Methionine sulfone, $HOOCCH(NH_2)CH_2CH_2SO_2CH_3$	Sulfate, aq, buffer pH 7.0	2480.0		1.0	
	Thiosulfate	Sodium thiosulfate pentahydrate, $Na_2S_2O_3{\cdot}5H_2O$	Sulfate, aq	2480.9	2472.5 2479.3	1.0	not used
	Sulfonate	Sodium methyl sulfonate, $NaCH_3SO_3$	DMSO, aq	2481.1		1.5	
	Sulfate ester	Sodium chondroitin sulfate, $C_{14}H_{19}NO_{14}SNa_2$	DMSO, aq	2482.7	2481.2	0.83	
	Sulfate	Sodium sulfate decahydrate , $Na_2SO_4{\cdot}10H_2O$	DMSO, aq	2482.4		2.2	not used

Sample Data Treatment

A sub-set of bulk soil samples and acetylacetone extracts from the physically protected S fractionation for the incubation experiment (Paper I) were freeze-dried and used for determination of S speciation by S K-edge XANES spectroscopy (Figure 6, section 4.3.3). The spectra were baseline corrected and normalized by setting the intensity at 2490 eV to 1.0. Thereafter, a least squares LCF regression method used the internally calibrated spectra of model compounds (Table 5) to fit the sample spectra. Model S species that contributed less than 2%, according to the preliminary fit, were excluded in the final fits. The quantification of contributing S species was through three groups of model compounds: reduced S (peak energy <2475), intermediate forms of oxidized S (peak energy 2475-2479), and highly oxidized S (peak energy >2479) (Table 5). The division corresponded to the three main absorption peaks in the sample spectra (Figure 8). The fit in the post-edge region was used as an internal quality control to ensure the model compounds included in the fit were representative of those in the sample.

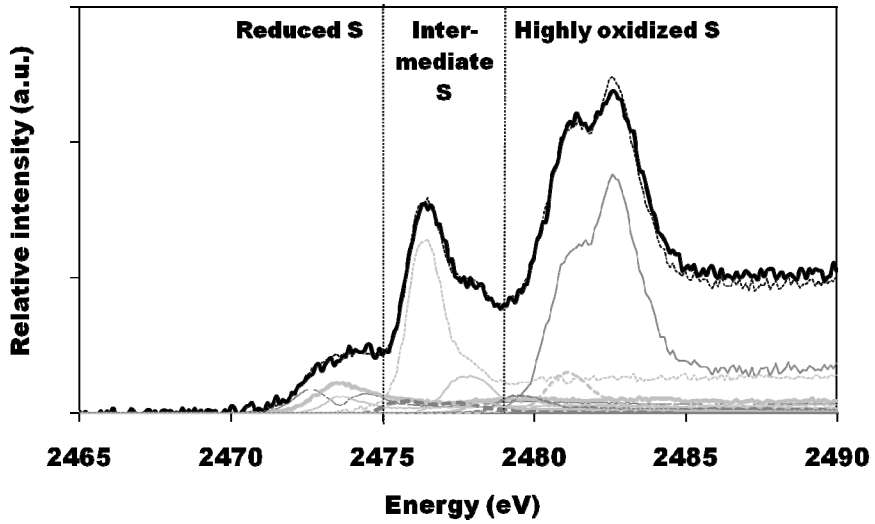


Figure 8. Sulfur K-edge XANES spectrum for Högåsa FYM bulk soil (black line) with best LCF fit (dashed black line) of model spectra (lines as described in Table 5). The divisions of model spectra into groups depending on their electronic configuration are depicted by dashed vertical lines.

4.3.6 Statistical Analyses

The differences between treatments and soils were investigated at a 95% confidence level with one-way and two-way analyses of variance (ANOVA), followed by paired t-tests between treatments within soils, and between soils within treatments. One-way ANOVA was used throughout Paper I (due to an imbalanced data set caused by a failed incubation tube) and for S treatment effects in Paper II. Two-way ANOVA was used for management system and soil effects in Paper II. Single linear regression and partial least squares regression (PLS) analyses were used to determine relationships between different soil properties and net S mineralization in Paper I. MINITAB release 15.1 was used for all statistical analyses, except PLS, where SIMCA-P version 11.0 was used. The fitting of the XANES spectra in Papers III and IV was done in Microsoft Excel with the LINEST function.

5 Results and Discussion

5.1 Soil Sulfur Cycling

Sulfur mineralization was higher than S immobilization in all cases, when no mineral S was added to the soils, resulting in net S mineralization in all tubes during the incubation (Paper I) and all pots within the –S treatment during the pot trial (Paper II) (Table 6). Furthermore, averaged over all sites, net S mineralization rate was higher in the FYM treatment than in the CR treatment, both in the incubation experiment (p=0.012) and in the –S treatment in the pot trial (p<0.001). With the addition of mineral sulfate (+S treatment in the pot trial, Paper II), transformation rates based on S uptake varied arbitrarily (Table 6); net S immobilization occurred in five out of eight cases and net S mineralization in the remaining three cases. There was no significant difference in the rate of S uptake between the management systems in the +S treatment, suggesting that when mineral S was applied, the uptake rate depended on factors other than S supply.

For individual sites, the difference between FYM and CR was only significant (p<0.05) in the –S treatment of the pot trial, and not at all in the Högåsa soil. There were some differences between the soils (Table 6), especially within FYM; in particular Orup FYM had a higher net mineralization rate than the other soils, especially in the pot trial. The conditions in the –S treatment of the pot trial clearly stimulated S mineralization in Orup FYM compared with the incubation experiment, whereas in the other soils, mineralization rates in the two experiments were similar regardless of management system. However, there was no correlation between the net S mineralization estimates in the two experiments; therefore, open incubation could not be validated as a prediction method for plant S availability in these soils.

Table 6. *Net sulfur mineralization rates (µg S per kg dry soil per day ± SE, n=3). Negative values indicate net S immobilization. Different lower-case letters within the same column denote significant differences between soils within that treatment (paired t-tests, p<0.05)*

	Incubation[†]		Pot trial[††]			
			-S		+S	
Soil	FYM	CR	FYM	CR	FYM	CR
Fjärdingslöv	48ac±1	50±10[†††]	33ab±1	24ab±1	-37a±20	36a±10
Fors	46a±1	40a±1	39a±5	24a±3	-32a±5	-30b±10
Högåsa	38b±1	37a±2	49b±5	48b±5	32b±8	-14b±16
Orup	59c±2	46a±3	107c±6	60ab±11	57ab±32	-4ab±5
Vreta Kloster	47abc±3	41a±0	Not included	Not included	Not included	Not included
Average	*48±2*	*41±1*	*57±9*	*39±5*	*5±15*	*-3±9*

[†]Calculated from accumulated SO^{4-}-S collected in leachates, Paper I

[††]Calculated from total plant S uptake plus net change (post-trial minus pre-trial) in soil sulfate pool in -S treatment, Paper II

[†††]n=2 due to clogging of one tube, soil not included in statistical analyses or total average calculations

The isotopic labeling of the original inorganic sulfate pool in the pot trial provided the possibility of tracing S from this pool as it moved through the soil-plant system (Figure 9) and calculate net S flow within the system (Table 7). The transformation rates estimated from isotopic S-flow (Table 7) were generally higher than the net calculations (Table 6), because they were closer to the gross processes. Both immobilization and mineralization occurred in all soils and S cycling rates were always considerably higher in pots containing soil from Orup FYM than in the other pots.

Table 7. *Net flow of soil S in the –S treatment in the pot experiment (µg S per kg dry soil per day)*

	Inorganic to plant S[a]		Organic to plant S[b] (mineralization)		Inorganic to organic S[c] (immobilization)	
Soil	FYM	CR	FYM	CR	FYM	CR
Fjärdingslöv	2	5	42	43	2	4
Fors	3	1	56	41	3	2
Högåsa	5	10	61	62	5	7
Orup	41	17	92	66	25	9

[a]Total ^{35}S activity in plant divided by specific activity of original sulfate in fresh soil

[b]Total plant S minus S from inorganic pool ([a])

[c]Total ^{35}S activity in organic soil fractions divided by specific activity of original sulfate

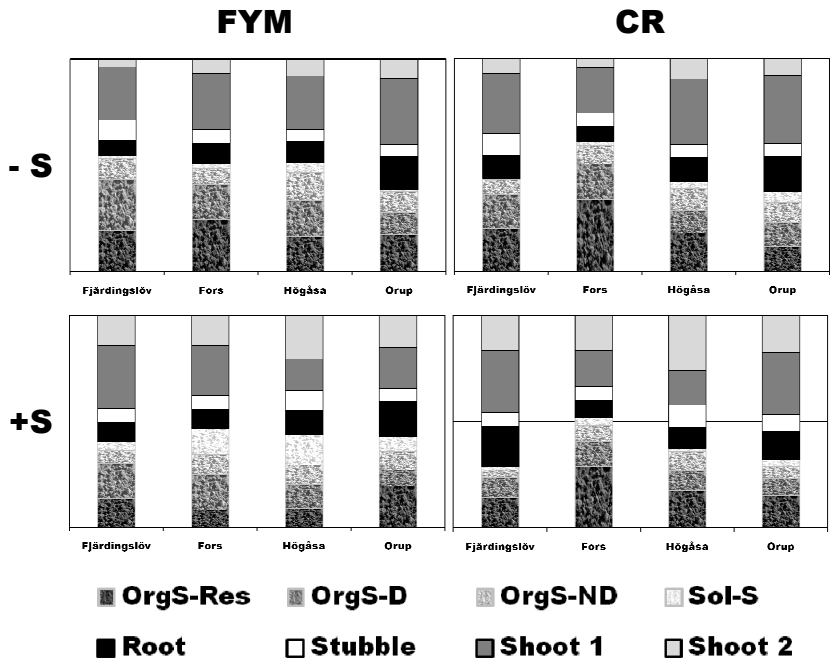


Figure 9. Relative distribution of initial inorganic sulfate within soil fractions (textured cells) and plant parts (solid cells) in the different soils after the pot trial (Paper II), calculated as total ^{35}S activity in a fraction divided by initial sulfate activity (isotopic decay accounted for). The line indicates 50% of initial labeled inorganic sulfate.

During the experiment, all fractions of soil organic S were involved in the S transformations, as indicated by the incorporation of isotopic ^{35}S into all pools (Figure 9), although net changes in pool sizes were generally small and insignificant (Figure 10). Furthermore, there was no correlation between net S mineralization and any organic S fraction (OrgS-ND, OrgS-D and OrgS-Res) (Paper I), suggesting small differences in transformation rates between the pools. However, the physically unprotected part of organic S in organomineral association (OrgS-ND) correlated positively with total S in biomass (p=0.009, R^2=70%, df=7) (Paper II) and had the highest average ratio of ^{35}S incorporation relative to total S in the organic fractions (Figure 11), suggesting a higher turnover rate in this pool. Conversely, the residual organic S pool had a disproportionately low incorporation of ^{35}S (Figure 11). Thus, there was some evidence for physical protection reducing S mineralization and the residual organic S pool having a slower turnover rate than the bulk of soil organic S (Ladd et al., 1993; Eriksen et al., 1998), albeit not to the extent previously considered. As the residual pool was only characterized by its hydrophobic nature (not extractable in acetylacetone), the pool was probably

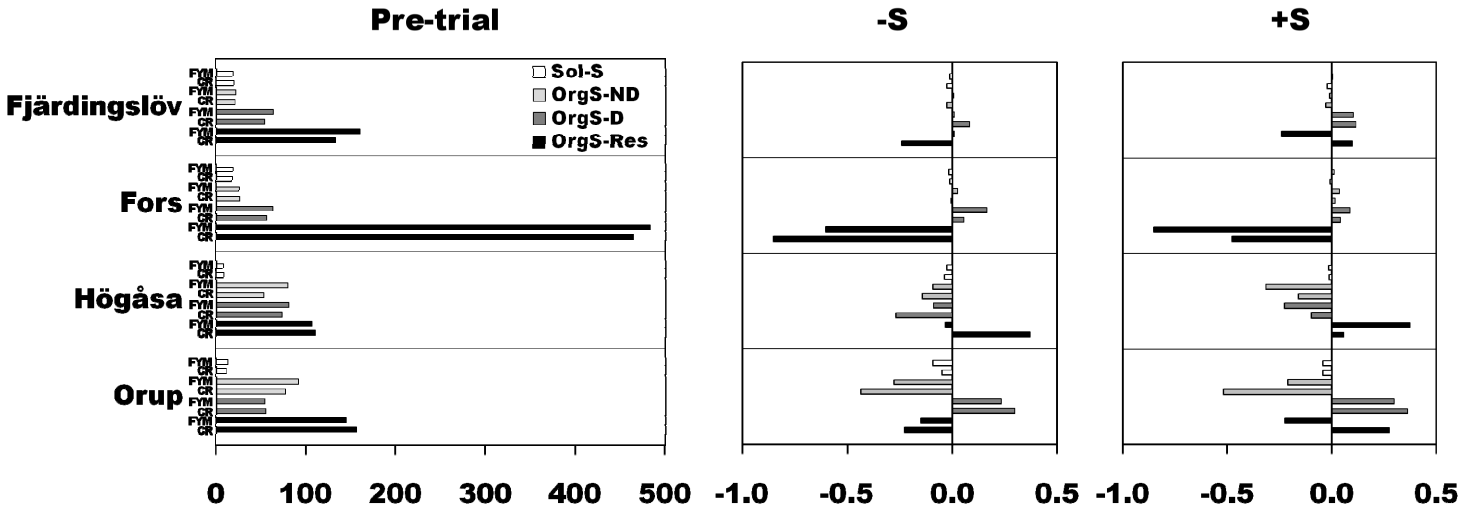


Figure 10. Total S (mg per kg dry soil) in soil fractions before the pot trial and average change rate (mg per kg dry soil per day) in the –S and the +S treatments.

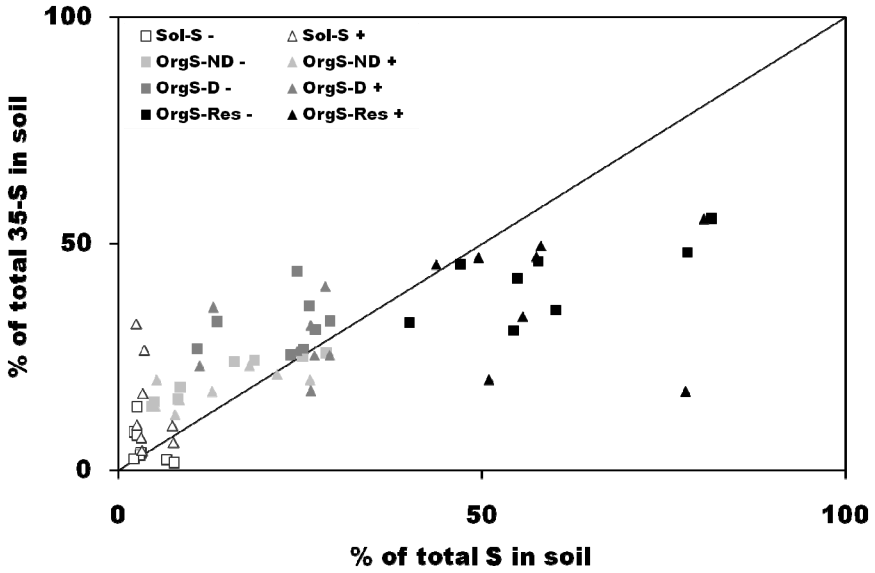


Figure 11. Percentage of total ^{35}S in soil recovered in each soil fraction after the pot trial plotted against percentage of total soil S recovered in the same fraction. Squares indicate –S treatment and triangles +S treatment. The line indicates a 1:1 ratio. The average incorporation ratios over both treatments were 3.2 for Sol-S, 1.8 for OrgS-ND, 1.5 for OrgS-D, and 0.7 for OrgS-Res.

heterogeneous with varying degrees of activity. The active part most likely consisted of microbial S and fresh organic matter, such as recently dead microbes, plant roots, and fresh plant litter.

The inorganic and easily dissolved organic S pool (Sol-S) was the only soil S pool with a net change during the pot trial, according to paired t-tests over all sites and both management systems within S treatment (p<0.05) (Figure 10). Evidently, this pool was being depleted in both S treatments, meaning that the rate of plant uptake of inorganic sulfate was higher than the mineralization rate. The PLS analyses (Paper I) revealed a negative relationship[4] between Sol-S and net S mineralization (Figure 12), suggesting S mineralization was stimulated when Sol-S availability was low. This implies biochemical mineralization, directly aimed at providing S to the microbes. In addition, PLS indicated net S mineralization was positively related to total N content and negatively related to C/N ratio and total C (Figure 12), suggesting mineralization through the biological pathway or, at least, co-mineralization

4. In a PLS loading scatter plot, correlations between variables are indicated by the angle between lines drawn from the variables to the origin: 0° - positive relationship, 180° - negative relationship and 90° no relationship.

with N. Thus, both mineralization pathways were active in the soils, although biological mineralization was probably dominant, as C/S ratios were within the 57-85 range normally found in microbial biomass[5] (Scherer, 2001).

The single regression analyses (Paper I) revealed no significant correlations between any basic soil property (Table 4, section 4.3.1.) and net S mineralization. However, total C, C/N ratios and microbial activity (as indicated by anaerobic N mineralization) were higher in FYM than in CR in all soils, although the differences were not significant. This may partly explain the higher S cycling rates in FYM, even though the data did not provide statistical support for it.

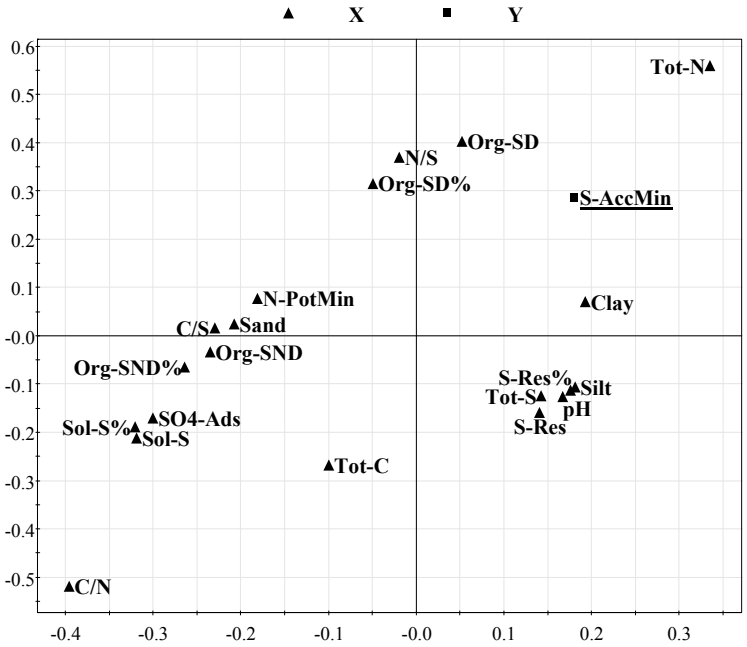


Figure 12. Loadings scatter plot for the Partial Least Squares regression. Variance in the X-variables (soil variables) was used to explain the variance in the Y-variable (S-AccMin, accumulated net S mineralization). Orup FYM data was excluded from the analysis, as this soil was identified as an outlier (Paper I). Variables as described in the text, with the addition of the following: N-PotMin = anaerobic N mineralization (microbial activity), SO4-Ads = initial adsorbed sulfate and S-Res = OrgS-Res. Sol-S%, Org-SND%, Org-SD% and S-Res% all refer to the percentage of total S recovered in the respective fraction.

5. As a substantial part of the C ingested by microbes is respired, the consumption ratios can be expected to be considerably higher than the incorporation ratios, resulting in mineralization of the surplus S.

5.2 Sulfur Supply and Plant Growth

Sulfur mineralization was vital for plant S supply in all treatments and soils (paper II). The isotope analyses revealed at least 70% of S uptake by ryegrass originated from the organic S pool, even when "optimal" amounts of mineral S were applied (+S treatment). Moreover, ^{35}S was recovered in shoots from the 2nd harvest in the –S treatment (Figure 9, section 5.1), indicating re-mineralization of recently immobilized S contributed to plant S uptake in all soils from both FYM and CR. However, the mineralization rates were too slow to avoid S deficiency in the ryegrass when no mineral S was applied. The S content at 2nd harvest was below the limit for S deficiency[6] in all shoots from the –S treatment and there were visual signs of S deficiency (yellowish, stunted shoots) in the ryegrass grown in all –S pots except those containing soil from Orup FYM (Figure 13). In the +S treatment there were no indications of S or other nutrient deficiencies, and the ryegrass appeared healthy (Figure 13). The S deficiency led to reduced biomass production ($p<0.001$) in the –S treatment, compared with the +S treatment (Figure 14). Analogous with the higher S cycling rates, FYM pots within the –S treatment had higher biomass production ($p=0.022$) and higher total plant S uptake ($p=0.003$, Figure 14) than CR pots. Within the +S treatment, only biomass production differed between FYM and CR ($p=0.011$), and in this treatment, biomass production was highest in the CR pots. Site differences in biomass production and total S uptake were significant ($p<0.001$) within both S treatments, as determined by ANOVA. The paired t-tests revealed some significant differences ($p<0.05$), especially in the –S treatment (Figure 14). The differences in S supply between the management systems and soils appeared to be accentuated by S deficiency, whereas, with sufficient S, other factors became more important for S mineralization.

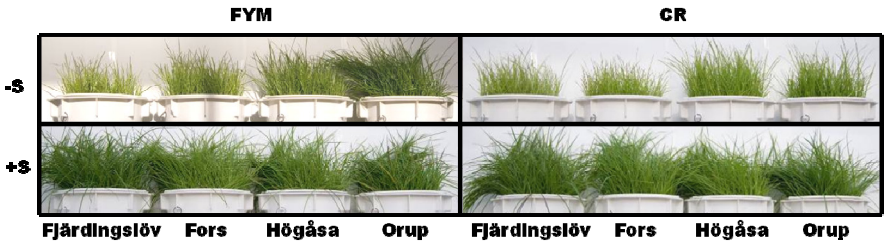


Figure 13. Ryegrass at 2nd harvest.

6. According to critical relationship calculations between total S and N as suggested by Mathot et al., 2009; data not shown.

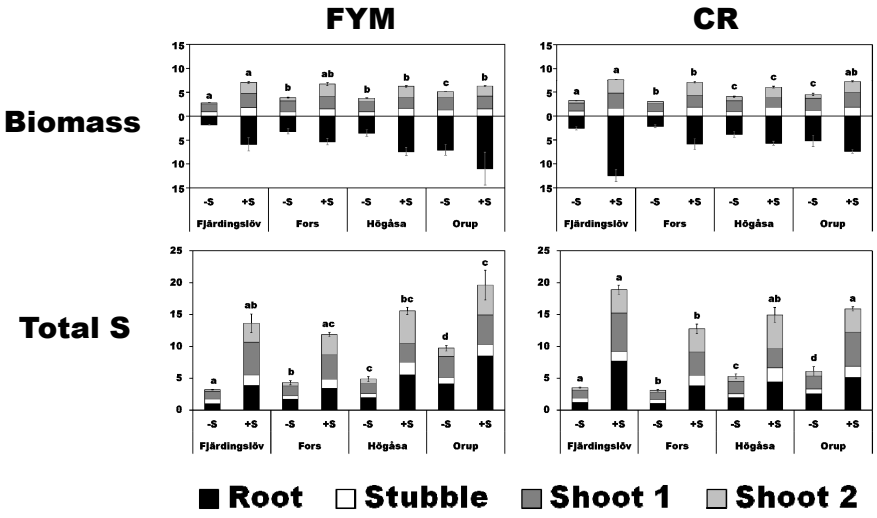


Figure 14. Ryegrass biomass production (g dry weight per kg dry soil) below ground (below x-axis) in roots, above ground (above x-axis) in shoots and stubble, and total S uptake (mg S per kg dry soil) in biomass. Error bars represent standard error for total above-ground biomass (shoots and stubble), root biomass and total S uptake. Different letters within the same treatment combination (FYM/CR *and* –S/+S) denote significant differences between the sites within that treatment combination.

5.3 Sulfur Speciation

The S K-edge XANES data treatment method developed for quantification of S species in soils and other natural samples (Paper III), provided reliable results for all bulk soil samples and extracts (Paper IV). The fits were generally good throughout the entire edge and post-edge regions, as exemplified by the Högåsa FYM bulk soil spectrum in Figure 8 (section 4.3.5). As previously suggested by others (Prietzel et al., 2003; 2007; Waldo et al., 1991), the separation between reduced S species was difficult. However, in combination with separate measurements of the inorganic sulfate content, the new method produced reliable separation between organic sulfate (sulfate ester) and other highly oxidized S species, due to a clear pre-peak/shoulder in the sulfate ester spectrum (Figure 3c, section 3.2.2). Furthermore, the internal calibration of model compounds revealed the relative absorption intensity was considerably lower for ester sulfate than for inorganic sulfate in relation to DMSO (Table 5, section 4.3.5). This has strong implications for the relative quantification of sulfate esters in soils and, thus, the interpretation of data, which emphasizes the importance of using internally calibrated model spectra to fit soil spectra.

Sulfate ester was the most common S species (40-64% of total S in bulk soil) in all samples (Figure 15), which resulted in highly oxidized S being the largest group (44-70% of total S in bulk soil), especially in the acetylacetone extracts (60-85% of total S in extract). As a group, reduced S species contributed the least (13-21%) to total S in all bulk soil samples, except for Orup FYM, where intermediate forms of oxidized S were least common (10%). The XANES analyses of the acetylacetone extracts and calculated extractability ratios (the percentage of a species group in bulk soil extracted by acetylacetone divided by the percentage of total S extracted) indicated that stabilization of organic S by organomineral association predominantly affected highly oxidized S species (extractability ratio >1) (Figure 16, Table 8), which was in accordance with previous reports on over-representation by HI-reducible S in clay-associated pools (Keer et al., 1990; Eriksen, 1996). Intermediate forms of oxidized S had a low extractability ratio (<1) and were mainly associated with the residual fraction. In the Högåsa soil, the speciation of the physically protected and unprotected organomineral fractions was almost identical; whereas, in the Fors soil, the highly oxidized species were less dominant in the unprotected fraction. The effect of management system on soil S speciation also differed among the soils and soil type appeared important for treatment response of S speciation.

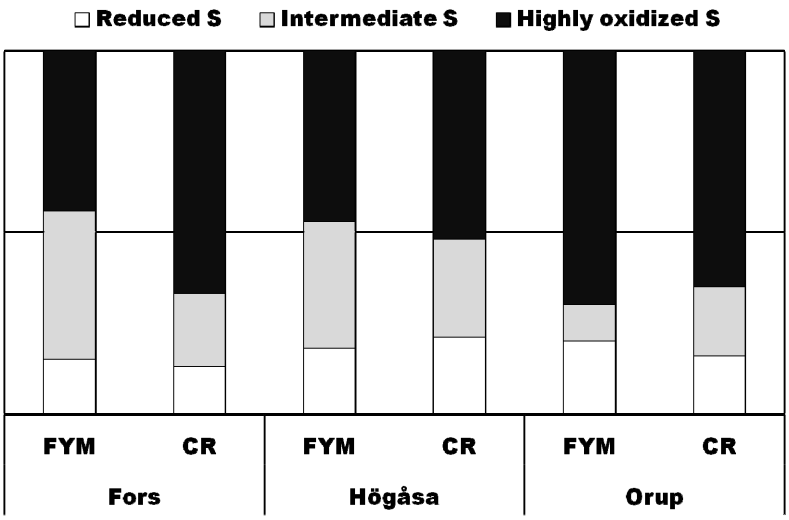


Figure 15. Relative distribution between groups of S species in bulk soil according to the LCF best fits of S K-edge XANES spectra. The horizontal line indicates 50% of total S in bulk soil.

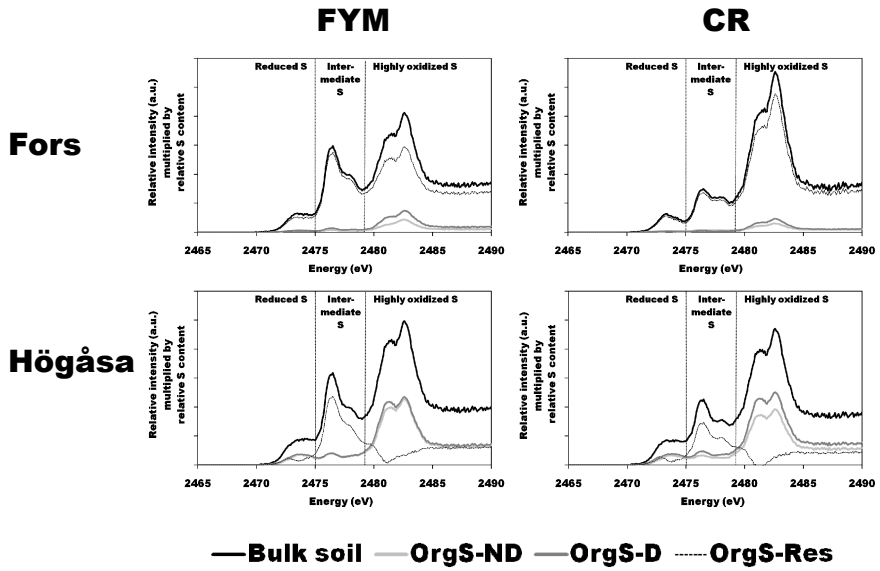


Figure 16. Modeled (best linear combination fit) S K-edge XANES spectra for bulk soil and acetylacetone extracts of organic S fractions in organomineral association (OrgS-ND and OrgS-D). The fluorescence intensity for each spectrum has been multiplied by the total S content in that fraction. The spectrum for the residual organic S fraction (OrgS-Res) was calculated as the difference between the bulk soil spectrum and the two acetylacetone spectra.

Table 8. *Distribution of different S forms (% of total S in extract) in the acetylacetone samples and ratios between the percentage of a species group in bulk soil extracted by acetylacetone and the percentage of total S extracted (extractability ratio)*

		FYM			CR		
Soil	Fraction	Reduced S	Inter-mediate S	Highly oxidized S	Reduced S	Inter-mediate S	Highly oxidized S
Fors	OrgS-ND	21	12	67	23	16	61
	OrgS-D	10	14	76	7	8	85
	Extractability Ratio	*0.9*	*0.3*	*1.7*	*1.0*	*0.6*	*1.1*
Högåsa	OrgS-ND	17	10	73	18	8	74
	OrgS-D	19	8	73	18	10	72
	Extractability Ratio	*1.0*	*0.3*	*1.6*	*0.9*	*0.3*	*1.4*

6 General Discussion and Conclusions

In this thesis, the main sulfur source for arable crops was the organic S pool in the soil (Table 7), even when inorganic S fertilizer was applied. Thus, in order to optimize S supply to crops, it is necessary to understand the dynamics of S transformations within the soil and the influence of management system, organic matter quality, and soil type. This thesis demonstrates that a livestock management system including ley and regular farmyard manure application increased the rate of S delivery to crops in the long term, compared to an arable crop production system, where the only organic matter input is incorporation of crop residues. Mineral S fertilization stimulated S immobilization, but plant uptake of S originating from organic sources was still higher than without mineral S application, due to increased biomass production. However, the extent of treatment effects depended on soil type. One of the soils, Orup, responded more to the management systems than the other soils did. In the livestock system, the S cycling rates in the Orup soil were considerably higher than in the other soils, whereas in the arable crop system, there was no distinct difference between the soils. Consequently, Orup FYM responded less markedly to inorganic S fertilization and the effects of S deficiency were less severe.

However, the reasons for the different behavior of the Orup soil were not clear. Differences in soil properties did not correlate with differences in S cycling rates, and the microbial activity, as indicated by anaerobic N mineralization, was at the lower end of the range in Orup FYM. As OrgS-ND was highest in Orup FYM and also correlated with total plant S uptake, physical protection of organic S could perhaps explain the lower mineralization rates in the other soils, but this would need to be confirmed through further

studies including more soils. It is possible the addition of P in the experiments[7] stimulated the microbial community (Cleveland et al., 2002) more in the Orup soil than in the other soils. At least, the P deficiency for crops due to long-term lack of mineral P fertilization in the field is most severe in Orup (Carlgren and Mattsson, 2001).

This thesis presented the opportunity to utilize and further develop relatively new methods for determining soil S chemical properties and organomineral associations. Thus, the organic S speciation was successfully determined in bulk soils and acetylacetone extracts with a new method for data treatment of S K-edge XANES spectra. This revealed relevant information about differences in soil S chemistry in that the effect of management system on soil S speciation differed between the soils and the majority of S in organomineral complexes was highly oxidized. Moreover, the lower contribution from S species of intermediate oxidization in Orup FYM compared with the other soils might be part of the explanation for the high turnover rate in this soil. In accordance with previous results (Vairavamurthy et al., 1994) intermediate forms of oxidized S were not extracted by acetylacetone to the same extent as the other groups of S species, but it is unclear whether the association with the residual S pool automatically implies a slower turnover rate. The residual S pool was the least active in the S cycling processes, but was definitely not passive. Schroth et al. (2007) proposed that intermediate forms of oxidized S are a transient group of compounds in soils, and sulfonates[8] are quantitatively important biochemical substances, including taurine, cysteic acid and sulfolipids (Vairavamurthy et al. 1994). Thus, a large part of the intermediate forms of oxidized S can be assumed to reside in microbes, plant roots and fresh organic matter, i.e. the active part of the residual S fraction. Therefore, the low contribution from S species of intermediate oxidization in Orup FYM was most likely an effect rather than a cause of the high S cycling rates.

7. P was added by leaching with KH_2PO_4 in the incubation experiment and was part of the initial fertilization added to all pots in the pot trial.

8. Sulfonates were the dominant species in the group of intermediate forms of oxidized S.

7 Implications and Future Perspectives

The focus of this thesis was on the influence of management systems on S cycling in Swedish arable soils, and the role of S chemistry, organomineral association, and physical protection. The development of a new method for data treatment of S K-edge XANES spectra rendered reliable quantification of S species in soils and soil extracts. This is a very important step towards an improved understanding of the chemical influence on soil S transformations, especially as it offers the possibility of determining S speciation in organic matter stabilized by organomineral associations and physical protection. These processes are thought to affect S mineralization rates, although there was little evidence of this in the data collected for this thesis. Differences in S cycling patterns were identified between soils and management systems, but it was not possible to elucidate the reasons for these differences based on the data collected. Therefore, soil chemistry, or at least S chemistry, was not the primary controlling factor for S mineralization. Future research should encompass more disciplines, especially microbiology, soil biology and plant physiology; and sulfur should be studied in relation to the cycling and speciation of other nutrients. For example, the importance of organomineral association and physical protection might be elucidated if there is sufficient information on the speciation of C and N in these pools, as the majority of S mineralization in this thesis appeared to be biological, i.e. a byproduct of C and N turnover. As P deficiency could be partially responsible for the observed differences in S mineralization rates between soils, this emphasizes the importance of simultaneous studies on macro- and micronutrients.

The differences in treatment response between the soils highlight the importance of including many soil types within treatments in order to isolate treatment effects from soil effects. In this respect, the Swedish long-term fertility field experiments are an excellent asset and deserve to receive more attention.

The practical implications of the results presented in this thesis are mainly for future research, but a few words should be added for farmers. Optimal biomass production requires that sulfur demand in the crop is satisfied, which in most Swedish arable soils necessitates input of S through mineral and/or organic fertilization. In the experiments presented in this thesis, S mineralization was elevated in a livestock production system compared to an arable crop production system, probably because the application of farmyard manure stimulates the S cycling processes. Although it remains unclear what caused the stimulation, farmyard manure can be a means to limit the dependency on mineral fertilizers, for example in organic farming.

8 Sammanfattning (Swedish Summary)

Svavel är ett viktigt näringsämne, men var under lång tid nästan förbisett ur växtnäringssynpunkt. Sedan 1980-talet har intresset för svavelomsättningen i åkermark ökat markant, som en följd av att svavelbrist började uppstå i jordbruksgrödor i västra Europa och Nordamerika. Svavelbristen var i sin tur en följd av att åtgärder efter debatten kring surt regn ledde till kraftigt reducerad svaveldeposition, samtidigt som nya kväve- och fosforgödselmedel introducerades. De gamla gödselmedlen innehöll ofta stora mängder sulfatsalter (≤ 24% S) från framställningsprocessen, men de nya medlen innehåller mycket lite svavel (≤ 2%). Dessutom har det ökade intresset för ekologisk odling medfört ett större behov av kunskap kring organiska gödselformer och möjligheten att utnyttja markens förråd av organiskt svavel. Växter är i princip uteslutande beroende av oorganiskt sulfat för att tillgodose sitt svavelbehov, men i normala fall är mer än 95% av svavlet i marken i organisk form. Mycket forskning har därför bedrivits med syfte att på ett enkelt sätt kunna förutsäga mängden svavel som tillgängliggörs för grödan från markens organiska material under en odlingssäsong. De metoder som har använts har dock varit otillräckliga i sin precision och tillförlitlighet.

I denna avhandling har relativt nya metoder använts för att kartlägga svavlets omsättning och kemiska egenskaper i jord från odlingssystemen djurhållning och växtproduktion på fem olika platser i en serie svenska långliggande bördighetsförsök. Den viktigaste skillnaden mellan systemen är det tillförda organiska materialet, som består av kreatursgödsel respektive skörderester. En inkubationsstudie och ett kärlförsök, där det oorganiska sulfatet märktes med ^{35}S, visade att kreatursgödseln medfört en ökning av svavelomsättningen jämfört med skörderesterna. Detta var speciellt tydligt i en av jordarna, Orup. Mineraliseringen av svavel var inte tillräcklig i någon av jordarna för att tillgodose behovet av svavel hos rajgräs, när ingen

svavelgödning tillfördes. Effekterna av den uppkomna svavelbristen var något mindre i kreatursledet, speciellt i fallet Orup.

Organiskt S kan stabiliseras i marken genom bindning till lermineral och dessutom skyddas fysiskt från mikrobiella angrepp genom inneslutning i mikroaggregat. Omfattningen av dessa stabiliseringsprocesser studerades genom extraktion med acetylaceton och dispergering genom ultraljuds-behandling. Mängden svavel i de olika fraktionerna varierade mellan jordarna, men endast det fysiska skyddet visade en signifikant negativ inverkan på svaveltillgängligheten för rajgräs. Inga andra korrelationer fanns mellan markkemiska egenskaper och svavelomsättningen, utom att en högre initial sulfathalt och en ökande C/N-kvot verkade minska mineraliseringen av S. De observerade skillnaderna i svavelomsättning kunde därför inte på ett tillfredsställande sätt förklaras med de variabler som studerades.

En ny metod för behandling av data från röntgenabsorptionsspektroskopi (XANES) utvecklades som en del av avhandlingsarbetet. Metoden tillät tillförlitlig identifiering och relativ kvantifiering av olika organiska och oorganiska svavelföreningar i jordprover och extrakt. Resultaten visade på skillnader i svavelkemi mellan olika jordar och odlingssystem, men eftersom behandlingseffekterna varierade mellan jordarna var det inte möjligt att dra några generella slutsatser kring odlingssystemens påverkan på fördelningen mellan olika svavelformer eller svavelformernas påverkan på omsättnings-hastigheterna. Analys av extrakt av fysiskt skyddat organiskt svavel, samt svavel som stabiliserats genom komplexbildning med lermineral, visade att högoxiderade former av svavel, framförallt sulfatestrar, dominerade i dessa fraktioner. Svavelformer med intermediär oxideringsnivå, mest sulfoxider, återfanns framförallt i den icke-extraherbara residualfraktionen. Omsättnings-hastigheten i denna fraktion har tidigare ansetts vara nästan försumbar, men enligt resultaten som presenteras i avhandlingen, deltog den aktivt i omsättningsprocesserna, om än i mindre utsträckning än övriga fraktioner. För att kunna avgöra vad fraktionernas olika svavelkemi har för betydelse för svavelomsättningen krävs att extrakt från fler jordar analyseras.

Resultaten visade att kreatursgödning har en stimulerande effekt på svavelomsättningen, även om det förblir oklart varför. Den nya metoden för kvantitativ bestämning av svavelkemin i jord och jordextrakt genom XANES spektroskopi ger goda förutsättningar att statistiskt undersöka sambanden mellan marksvavlets kemiska egenskaper och dess omsättning. Avhandlingen tydliggör vikten av att använda långliggande försök, att inkludera många olika jordtyper i samma behandling för att kunna särskilja behandlingseffekter från jordeffekter, samt att studera flera näringsämnen samtidigt och arbeta mer tvärvetenskapligt inom markkemi och markbiologi/mikrobiologi.

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