Decomposition and nitrogen transformations in digested sewage sludge applied to mine tailings-effects of temperature, soil moisture, pH and plants

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Abstract

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Applying sewage sludge to mine tailings to encourage growth of vegetation in order to prevent environmental problems such as erosion and leaching of metals began around 1970. Use of sewage sludge for mine land reclamation is today an acceptable area of application and is in many cases preferable to spreading sludge as an organic fertilizer on agricultural land, since many sludges contain metals and pathogens. The sludge has been shown to be favourable compared to other additives that do not contain organic matter with respect to improved soil properties and nutrient cycling.

The present work was conducted to elucidate the influence of temperature and soil moisture in an incubation experiment on the dynamics of decomposition and nitrogen (N) mineralization in digested sewage sludge mixed with pyrite mine tailings from the Aitik copper mine, Gällivare, Sweden. The data were fitted to a first order two-compartment model. The importance of pH and plants, barley (*Hordeum vulgare*) and red fescue (*Festuca rubra*) was studied in a greenhouse experiment. The experimental conditions (temperature, moisture and pH), were chosen to represent situations occurring under field conditions.

Temperature and soil moisture had clear effects on microbial activity in the tailings-sludge mixture and explained 93% of the variance in respiration rate constants. The proportions of carbon and nitrogen mineralized from the substrate during approximately 100 days, measured in the incubation and the pot experiment, were between 20 and 30% and 15 and 35% respectively. The C:N ratio was roughly constant (8-10) throughout the experimental periods. From these data, good estimates of the amounts mineralized during the first season under different climatic conditions can be obtained. Interestingly, the amounts mineralized N (net) in the pots were much lower than the determined changes in organic N, suggesting that large amounts were lost as gas, most likely via denitrification.

Key words: reclamation, mine tailings, sewage sludge, decomposition, nitrogen, temperature, moisture, model, pH and plants.

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Papers I and II

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Wennman, P. & Kätterer, T. The effect of moisture and temperature on C and N mineralization in mine tailings mixed with sewage sludge in a long-term incubation experiment. *(Manuscript)*

II. Wennman, P. & Ledin, S. Nitrogen transformations in sewage sludge mixed into mine tailings. *(Manuscript)*

Introduction

Awareness regarding environmental problems relating to surface mining increased during the beginning of the Twentieth Century. Hazards arising from the mine waste were, like today, degradation of the landscape, leaching of heavy metals, acidification of freshwater and groundwater and wind and water erosion (Gemmell, 1977; Bradshaw & Chadwick, 1980; Mays, Sistani & Soileau, 2000). The need for mine waste treatment became more and more obvious and reclamation by plant establishment began around 1940 in the United States (Plass, 2000) and in the early 1970s in Sweden (Lindgren, 1975).

Mine tailings constitute an extremely harsh environment for plants compared to natural soils. Growth and survival are limited by unfavourable physical, chemical and biological factors, including sand-blasting effects from wind eroded sharp-edged particles, drought due to low water holding capacity, lack of plant nutrients, occasionally high surface temperatures and low pH and metal toxicity.

In the early reclaimed mine spoils, inorganic fertilizers in combination with lime were added, in order to improve soil properties for plant growth. Grasses together with legumes (*e.g.* white clover, *Trifolium repens*) were the species mostly frequently used, with their advantages of fast initial cover of the spoil or tailings surface. However, fertilization in the absence of organic matter was not enough and often resulted in an inadequate and slow soil-plant ecosystem development, especially at acidic sulphide mine sites. Plant establishment with sewage sludge as a soil amendment was tested and was shown to be more successful than previous methods without organic matter. Application of sewage sludge to mine waste has been conducted as an acceptable reclamation method over the past 30 years (Haering, Daniels & Feagley*,* 2000).

During the year 2000, sewage sludge from Swedish treatment plants was land-filled (34%), used on agricultural land (21%), used on other land (32%) including mine land reclamation and used in other ways or temporarily stored (13%) (Sveriges officiella statistik, 2000). Since the year 2000, the use of sludge on mine land for reclamation has increased and on agricultural land it has decreased. Soil amendment, fertilization and mulching are the main reasons for the use of sewage sludge on mine land (Ledin, 1999).

High amounts of available nitrogen and phosphorus in the sludge and the beneficial influence on soil properties related to plant growth are the most important reasons for the use of sludge for mine reclamation by plant establishment. During decomposition of the sludge, large amounts of inorganic nitrogen and other nutrients are released and made available for plants, or further transformed in microbial processes. There are several factors limiting decomposition, whereof temperature and soil water are most important. Soil acidity may limit decomposition if pH drops below critical levels. A high buffering capacity is important when sludge is used as a soil amendment on sulphide mine tailings, since these tailings become acidified when exposed to oxygen. pH changes are thereby reduced and the uptake of nutrients and biochemical processes for soil organisms and plant roots can continue without reduction.

If sewage sludge is applied initially, with the aim of supporting growth over several years to come, the amount of sludge must be so large that plants can only absorb parts of the mineralized N during the first season. The rest of the transformed N may turn into N_2, but may also be lost as nitrate to groundwater or volatilized as dinitrogen oxide or ammonia and thereby pose a threat to the natural environment.

Background

The influence of organic matter for plant establishment on tailings

Plant growth and survival in sulphide mine tailings are restricted due to several factors. High levels of sulphide minerals such as pyrite may, when exposed to oxidation, decrease pH in the tailings and release toxic levels of metals (Ferguson & Erickson, 1988). Lack of plant available nutrients and organic matter in the tailings is obviously even more essential (Gemmell, 1977; Sikora *et al.,* 1982). Other factors such as drought, high surface temperature and wind erosion may also restrict growth but organic matter can change the situation in favour of plant growth.

Several studies have shown the influence of organic matter for successful establishment on sulphide mine tailings. The long-term effects (some years) are most important (Seaker & Sopper, 1988). Indeed, addition of chemical fertilizers and lime can maintain a plant community temporarily, but cannot improve soil properties and create a sustainable plant-soil ecosystem as is the case with organic amendments, *e.g.* sewage sludge, paper mill sludge, animal manure or peat. Organic matter can also be applied as a mulch to control erosion and weed growth and to conserve moisture.

Sewage sludge has been used as a soil amendment in agriculture and land reclamation purposes for more than 30 years and its effects on soil properties are well documented, and do not differ from those of other organic substrates. Specific studies on mine spoil reclamation with sewage sludge have been performed. Joost, Olsen & Jones (1987) observed decreased organic matter content and decreased aggregate stability with time. Bulk density was lower, but pH and buffering capacity increased compared to unamended sites. The buffering capacity in the sludge is of particular importance in reclamation of acidic spoils. Differences in buffering capacity and pH are not only determined by the amounts added, but also by the type of stabilization during treatment of the sewage water for precipitation of phosphate. The most common salts used are aluminium sulphate, iron sulphate, iron chloride and calcium hydroxide (Kirchmann, 1988). Stabilization by Ca-salts has been shown to increase pH in the sludge more than the use of other metal salts, *e.g.* Fe- and Al-stabilized salts (Sjökvist & Wiklander-Johansson, 1985; Soon, Bates & Moyer*,* 1978). Enhanced root distribution to deeper soil layers is another benefit observed in mine spoils amended with sewage sludge (Seaker & Sopper, 1988). Distribution of roots itself influences the mechanical stability by soil penetration and also enhances microbial activity in the rhizosphere by release of root exudates (Uren, 2001).

Temperature, moisture and pH as factors affecting microbial activity

Climatic factors, *i.e.* temperature and precipitation influencing soil moisture, are the most important factors for mineralization rates in most soils (Stanford & Epstein, 1974; Kirschbaum, 1995; Antonopoulos, 1999). Both chemical and biological reactions proceed faster at high temperature (Waksman & Gerretsen, 1931; Kirschbaum, 1995; Lomander, Kätterer & Andrén*,* 1998a). Nitrogen mineralization, *i.e.* ammonification, has been shown to continue to a considerable range above 35 ˚C (Harmsen & Kolenbrander, 1965). However, in nature the activity is normally reduced at these high temperatures due to limited water availability.

At temperatures below 0 ˚C, the microbial activity is low and restricted to availability of unfrozen water occurring in the smallest micro pores, which can keep water in liquid form at low matric potential. Indeed certain bacteria, *i.e.* psychrophiles adapted to cold environments, *e.g.* tundra climate, have the ability to decompose organic matter down to -7 ˚C (Flanagan & Veum, 1974).

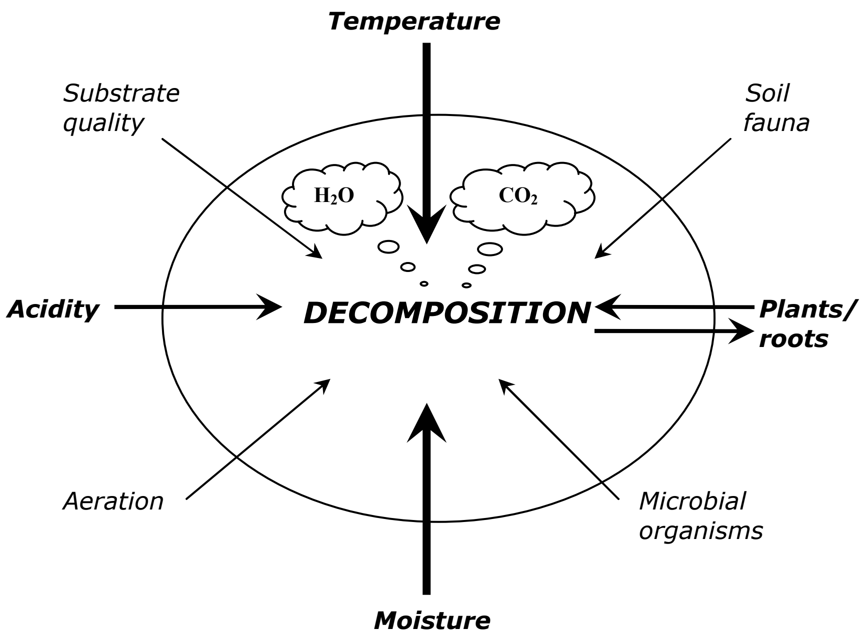

Fig. 1. Schematic view of the most influential biotic and abiotic factors affecting decomposition of organic matter, such as sewage sludge. Factors with bold text were studied in this work where arrows indicate impact and direction. Naturally there are several interactions among these factors.

Soil water and microbial activity often show a linear relation up to optimal moisture levels around 1.5 MPa (Stanford & Epstein, 1974), but other functions, such as log linear relationships, also describe the moisture response on decomposition (Lomander, Kätterer & Andrén, 1998b). At moisture conditions close to the wilting point or at water saturation, the activity decreases rapidly. Microbial activity due to water consumption is regulated in a direct way in the metabolism, through hydrolytic reactions and in cell respiration, and in an indirect way where water acts as a medium in which molecules, ions and gases are transported (Jansson, Johansson & Alvenäs, 1989). Specific microbial processes, *e.g.* movement of protozoa, nitrification, bacterial growth or fungal growth, are often limited to the thickness of the water film on aggregates corresponding to a certain water potential. The groups of microorganisms responsible for these processes are exposed to water stress when the thickness is below a critical level (Harris, 1981).

Soil pH or soil acidity is considered to be a key factor for microbial activity. Both C and N mineralization are affected positively if pH is increased as a result of liming acid soils (Andersson & Nilsson, 2001) or lowering pH in neutral soils by adding sulphuric acid (Fu & Tabatabai, 1987). At the same time, however, mineralization occurs over a broad range of pH levels, without a marked reduction in activity. The explanation is that single groups of species in a diverse population of decomposers, all with different pH optima, can be replaced as pH values deviate from their pH optima. For instance the number of bacteria and actinomycetes is decreased at low pH whereas fungi are more abundant as a result of less competition. However, when organisms are exposed to pH stress, it can be a direct effect of increased hydrogen ion concentration or a secondary effect of toxicity from other elements or molecules (Alexander, 1980).

Microbial activity in the rhizosphere, the immediate zone close to the root hairs, is far more important than the activity in the bulk soil (Pinton, Varanini & Nannipieri, 2001). A natural effect of root activity is decreased soil moisture content as an effect of water uptake, which in turn slows down microbial activity. Root distribution influences soil moisture and aeration via changes in soil structure. Other effects are more related to the chemical environment regulated by excretion of compounds from the roots, *i.e.* rhizodeposition. Deposition of C-rich compounds, mostly occurring from growing roots, increases the C pool in the rhizosphere and hence the C:N ratio. The effect can be reduced N mineralization or even a net immobilization (Janzen & Radder, 1989). However, the effect of C lost from the roots on net N mineralization is contradictory and may also lead to higher amounts of N being available for roots due to stimulation of the microbial populations (Clarholm, 1985). As much as 40 % is estimated to be lost of the plants primary C production through rhizodeposition (Brimecombe, De Leij & Lynch, 2001).

History of the field site

At Aitik, Gällivare (67˚0.06'N; 20˚0.8'E) in northern Sweden, every year since 1997 field experiments have been performed for determination of yield and C and N mineralization. Different soil amendments, mostly sewage sludge, as well as different plant species and management practices have been used. Further reading about soil amendment and plant establishment on mine tailings can be found in Ledin (1999) and Barnhisel, Darmody & Daniels (2000).

Objectives

The overall aim of this work was to determine the influence of temperature and moisture for the decomposition dynamics of digested sewage sludge mixed into pyrite mine tailings in an incubation experiment and to examine how plants and pH affect microbial activity and nitrogen mineralization in the mixture through studies in a pot experiment.

Materials and methods

Copper mine tailings and digested sewage sludge

Mine tailings from the field site at Boliden copper mine Aitik, were mixed with digested sewage sludge from the sewage treatment plant at Henriksdal, Stockholm. The mixture represents a homogeneous substrate for plant growth and microbial turnover. The proportion of sludge used was 20% by volume and approximately 6% by weight on a dry matter basis, and was similar for both the incubation and the pot experiment.

The tailings produced at the Aitik copper mine were moderately oxidized and the sulphur level was determined to around 2.5% and pH was 4.2 at the start of the experiments. Sulphur concentrations are primarily dependent on the pyrite content and vary between 0.6-4.75% by weight depending on from where in the tailings pond the material is taken. The tailings are a residual product of crushed ore and consist mainly of quartz, feldspar, plagioclase, muscovite, biotite and traces of pyrite (FeS_2) (Abrahamsson, 1994, unpublished observations). The texture varies with location in the pond but fine sand is the dominating fraction. Silt- and clay-sized particles occur in small amounts and the texture can be classified as a silty loam.

The sludge from Stockholm Vatten used in this study had been stabilized by iron sulphate, dewatered to a water content of around 70% and anaerobically digested at a temperature of 33-37 ˚C according to the normal procedure in the sewage treatment plant. The sludge was transported from Stockholm to Gällivare and a sufficient amount was taken from the field at the mining site (Aitik) for preparation at the Department of Soil Sciences, SLU, Uppsala. The sludge material was stored at outdoor temperature from April to June for 6-8 weeks and then stored at -18 ˚C before preparation for the experiments.

As a result of the decomposition during the digestion treatment, the C:N ratio of the sludge was reduced to around 10, and thus was far below the critical ratio (20-25) when the organic nitrogen is used to a higher extent for anabolism of the microbial biomass (net immobilized), than used as an energy source (net mineralized). However, the C:N ratio of the organic matter is only a rough indicator of net mineralization. It is so because the rate of C and N mineralization is a mean value of the turnover rates of different forms of C and N in the residue, *e.g.* cellulose, sugars, proteins and fatty acids (Whitmore & Handayanto, 1997). Note that not only the C:N ratio, but also the microbial efficiency may differ in utilizing N for mineralization or immobilization in digested sludge, where dead microbial cells dominate, compared to N utilization in crop residues (Whitmore & Handayanto, 1997; Badalucco & Kuikman, 2001). Growth of populations of heterotrophic bacteria (lag phase), although the C:N ratio is less than 20, will likely result in a net immobilization (Fig. 4, Paper I).

Concentrations of different elements occurring in different types of digested sewage sludge vary considerably. The sludge used in the current experiments was roughly similar in N concentration and higher in ammonium and phosphorus concentration compared to other Swedish sludges, while its potassium content was lower. The pH values also show similarities reflecting the same type of precipitation chemicals used for phosphorus, where aluminium sulphate and iron sulphate salts are common. Not only chemicals used in treatment, but also quality of the sewage water and storage of the sludge have an influence (Adegbidi & Briggs, 2003). The sludge characteristics also change over the year as a result of the varying quality of the sewage water going into the treatment plant. In addition, the period of storage and type of treatment before soil application have an impact on the concentrations of different elements (Pierzynski, 1994; Bernal *et al.,* 1998). Availability of N for microbial growth and the potential for net N mineralization are therefore far from equal in different sewage sludges. In Table 1, nutrient concentrations of different types of sludges are compared using data from Sommers (1977); Sjökvist & Wiklander-Johansson (1985), and Stockholm Vatten (2002).

Table 1. *Characteristics of anaerobically digested sludge showing range and mean values (within brackets): A) Sommers (1977). Data from approximately 150 sewage treatment plants in United States, B) SLL (1985). Results from a Swedish survey in 1980/81, which included hundreds of treatment plants, C) Stockholm Vatten (2002). Henriksdal treatment plant range in monthly values for the year 2002, and D) Sludge analyzed for the present experiment, n=4*

Parameter	**Concentrations (g kg^{-1})**			
Sludge	**A**	**B**	**C**	**D**
C-org [A, B, D] Loss on ignition [C]	180-390 (276)	-	384-469 (426)	264-261 (262)
N-tot	5-176 (50)	5-110 (40)	12-43 (38)	33.7-34.3 (34)
NH_4-N	0.12-67.6 (9.4)	0.5-22 (4)	3-13 (10)	-
NO_3-N	0.002-4.9 (0.52)	-	-	-
P-tot	<50-143 (33)	7-45 (25)	36-43 (39)	37.6-39.8 (38.5)
K	0-26 (5.2)	1-7 (2.5)	1.0-1.7 (1.3)	-
S	8-15 (12)	-	8-10 (9)	-
Ca	19-200 (58)*	2-400 (20)*	15-23 (18)	-
pH	-	5-13 (7.5)	7.3-8.9 (7.8)	8.18-8.23 (8.2)

* High variation, depending on type of precipitation chemical used.

Experimental design

Incubation experiment

An incubation experiment running over 3 months (96 days), was set up for C- and N-mineralization, with determination of CO_2 evolution and accumulated mineralized N respectively at constant moisture contents and temperatures. These variables were combined at 4 different levels of moisture: 2, 8, 14 and 24% by weight (corresponding to 10, 39, 69 and 118% of water holding capacity determined after drainage of 100 g of saturated tailings-sludge mixture in a funnel with a fine mesh to equilibrium and drying thereafter for 24 hours), with 4 different temperatures: -1, 5, 10 and 15 ˚C, altogether 16 treatments with four replicates. Samples were incubated in an ES 500 incubator, Nüve, Germany.

Soil moisture contents were chosen to cover a range from beneath the wilting point up to expected optimal conditions for microbial activity, and temperatures were from a level with expected negligible microbial activity up to maximum temperature expected just below the soil surface in the field.

The experimental set-up for determination of mineral N included destructive sampling. The number of samples prepared was therefore higher compared to the number of CO_2 measurements, or compared to all combinations of moistures and temperatures multiplied by the number of sampling occasions, which in this experiment was five. Of course destructive sampling in a time sequence requires a homogeneously prepared substrate in order to show results of the process studied, rather than results due to the original variations in the substrate.

Pot experiment

In the pot experiment, barley *(Hordeum vulgare)* and red fescue *(Festuca rubra)* were used to study the influence of root activity on N mineralization. The fast growing barley consumes the dominant part of the nutrients. The pot experiment was carried out in a greenhouse from early September to mid December, (14 weeks), with a mean temperature between +12 ˚C and +15 ˚C, and with 7 treatments in 4 blocks, in a split-plot design. Treatments were as follows: 3 levels of pH (4, 5 and 5.5) in irrigation water applied to four pots with and without plants respectively, plus a control treatment, (pots with pure tailings irrigated with deionized water, pH 5.5).

By addition of sulphuric acid, the pH levels in irrigation water were chosen in order to achieve a chemical environment in the soil solution similar to that when pyrite is oxidized, and assumed to be representative of the variation that may occur at different pyrite levels in the tailings. The pot volume was 7 litres and the construction included a drainage pipe at the bottom from which drainage water was taken for determination of pH. Drainage water was recycled to the pots in order to minimize losses of nutrients.

Samples were taken at harvest with a soil corer made for pot sampling. From each pot, 4 sub-samples were taken through the soil profile to form a general sample, which was homogenized and stored in plastic bags at -18 ˚C prior to analysis. Plant material was harvested at flowering using grass shears. After drying at 38 ˚C, air-dried samples were weighed for yield determination and milled. Ear, straw and leaf of the barley plant were milled together.

Analysis and data treatment

Background and principles for decay models

A simple model for turnover of organic matter was used in order to predict the dependence on temperatures and soil moisture of the process during periods not covered in this experiment. After obtaining the estimated data from the model, curves fitted to temperature and moisture response can be extended in time or at higher temperatures and moisture levels by extrapolation.

The simplest model assumes a homogeneous substrate decomposing at a certain rate throughout the period of measurement. By adding one or more pools to the first pool, a two- or multi-compartment model is constructed. The more complex the substrate subjected to the model, the more pools have to be assumed for an accurate description of the decomposition. Multi-compartment models are often used when litter decomposition is described. Decomposition of a substrate occurs predominantly following first order kinetics, *i.e.* the substrate levels are limiting for the turnover rate, which is assumed to be proportional to the substrate concentration (Paul & Clark, 1996). Indeed other reaction types also occur, but are not described here.

A first order reaction can be mathematically described as:

$$\frac{dA}{dt} = -kA \qquad \text{eqn (1)}$$

where the amount of A during time t is turned over at the rate $-k$. Integration generates the following equation;

$$A_t = A_0 e^{-kt} \qquad \text{eqn (2)}$$

where A_t is the concentration of the substrate remaining at any time t.

In our experiment (Paper I), data from the measured CO_2-C fluxes obtained from incubation were fitted to a two-compartment model, following first order kinetics. Carbon mineralization was assumed to proceed from two independent substrate fractions with specific pool sizes, readily decomposable (α) and slowly decomposable ($1\text{-}\alpha$) degrading in parallel at two different turnover rates k_l and k_r (Jenkinson, 1977), with (α), ($1\text{-} \alpha$), k_l and k_r as input parameters,

$$C_{flux} = C_0\,[\alpha\, k_l \exp(-k_l t) + (1-\alpha)\, k_r \exp(-k_r t)] \qquad \text{eqn (3)}$$

A two-compartment model can also be used when the pools are assumed to interact with each other. This is the case when the refractory component is converted to the labile state (consecutive first order) (Andrén & Paustian, 1987). However, in this experiment changes in the number of pools or interactions did not increase R^2, and were therefore not used. The assumption of input parameters is determined by several factors, *e.g.* substrate quality, substrate quantity and inhibiting factors such as heavy metals (T. Kätterer, pers. com).

For description of model fitting by analysis of regression, for the model and for the temperature and moisture response functions, see Paper I.

Substrate (*tailings + sludge*) *and plant material*

Determination of dynamics of CO_2 evolution (C_{flux} mg g^{-1} substrate day^{-1}) in the tailings-sludge mixture during incubation was performed using NaOH as a carbon dioxide trap placed together with the incubated samples. The amount of CO_2 respired was measured by titration with HCl according to Stotzky (1965). Mineralized N (NH_4-N and NO_3-N) was measured colorimetrically on a TRAACS 800 auto-analyzer (Bran and Luebbe, Germany), after extraction with KCl. N mineralization was determined from accumulated mineral N during incubation and from initial organic N content.

In the pot experiment, total C and N soil samples (air-dried at 45 ˚C for 3 days) and plant N was analyzed on a LECO® CHN 932 analyzer. Changes in organic C and N were determined on dried samples by subtracting the amount C and organic N in the substrate at the end from the initial values (C-tot_{start} – C-tot_{end} and N-org_{start} – N-org_{end}). From the plant material, N absorption was calculated after measuring aboveground biomass production (including estimation of N accumulated in roots) and N concentrations measured on dried plant litter. Estimation of N accumulation in root biomass was calculated from the measured aboveground biomass and related to known N content in barley, root:shoot ratio≈0.2, according to Hansson, Pettersson & Paustian (1987).

Statistics

Data were treated using a randomized design in the incubation and a split-plot design in the pot experiment. Variance analyses (ANOVA) were performed using SAS procedure GLM (SAS Institute Inc., 1999). A non-linear regression analysis was performed to determine the variability between the modelled and the measured carbon data. For comparison of mean values, Students' t-test assuming equal variances were used. In the pot experiment, the dataset was also treated without outliers and compared for test of significance.

Results and discussion

Temperature and moisture effects on C_{fluxes}, comparisons between modeled and measured values (Paper I)

Both temperature and soil moisture clearly had effects on microbial activity in the tailings-sludge mixture. These variables explained 93% of the variance in respiration rate constants. During 14 weeks of incubation, significant differences were shown in mineralized carbon (C), ranging from 2% (at -1 ˚C and 2% moisture) to around 20% (at 15˚C and 24% moisture) of initial C content in the substrate. By studying the dynamics of C_{flux} (evolved mg CO_2-C g^{-1} substrate day^{-1}) it became obvious that the activity was more suppressed at 2% moisture compared to the three higher moisture contents (8%, 14% and 24%) (Fig. 1, Paper I). This was also shown in the temperature and moisture response functions, which were exponential and log linear shaped respectively (Fig. 2).

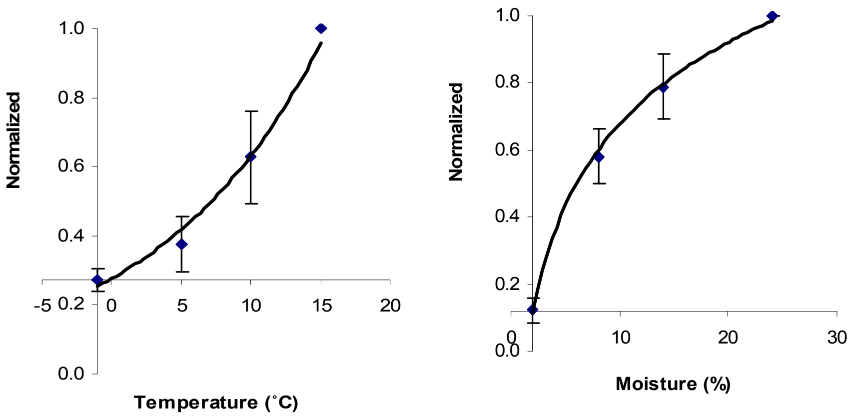

Fig. 2. Temperature and moisture response functions obtained from the incubation experiment. The highest temperature and moisture are reference values which the lower values are related to, and are set to 1.0 without standard deviation.

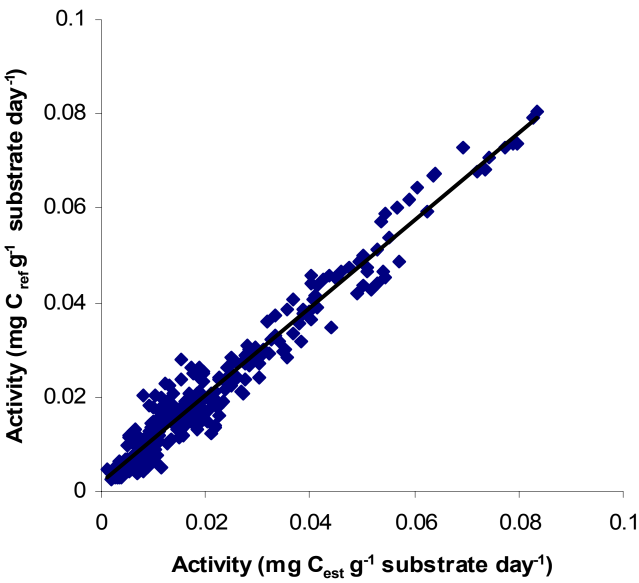

Fig. 3. C_{flux} (mg CO_2-C g^{-1} substrate day^{-1}) model values plotted against reference values, R^2=0.96.

The model, based on the obtained rate constants *(k)*, fitted with high accuracy, R^2=0.96, to the measured CO_2 evolution (Fig. 3). According to the model, during the first 15 to 25 days the decomposition rates were markedly higher than during the rest of the period, implying a relatively higher activity of microorganisms decomposing the readily decomposable carbon *(α)* than those decomposing the slowly decomposable carbon *(1-α)*. The C:N ratio in the sludge was 9 and reflects a situation in which most of the readily decomposable carbon, *i.e.* sugars, amino acids and short chain fatty acids, had already been turned over during the digestion process in the treatment plant.

Effects of temperature and moisture on net N mineralization (Paper I)

Net N mineralization was also shown to depend on changes in temperature and moisture, as expected. However, no clear trend over the treatments was found, *i.e.* significant differences were shown only between some of the treatments, above all at temperatures and moisture conditions where the activity was low or intermediate (Table 4, Paper I). The highest amounts of N mineralized (13.5% to 15%) were similar at 10 ˚C and 15 ˚C with 14% and 24% moisture. Zak *et al.,* (1999) found that 60-70% more N was mineralized over 16 weeks at 10 ˚C than at 5 ˚C, independent of soil moisture. Interpreting data from treatments where the lowest activity occurred was difficult, due to large standard errors. Possible explanations for this are: low mineralization/ammonification activity or simultaneous mineralization and immobilization resulting in negligible net effects on the direction of N flow and to some extent with the destructive sampling strategy.

Initially, most of the mineralized N was in the form of NH_4^+ but considerable amounts of NO_3^- were formed during the latter part of the incubation period. According to temperature demands for nitrifying bacteria, shown to be most important above 5 ˚C (Malhi & McGill, 1982), we observed formation of nitrate only at 10 ˚C and 15 ˚C after 20 days. The nitrification rate was indicated to be higher than the net mineralization rate, *i.e.* formation of NH_4^+ at 24% moisture as long as nitrate was formed (Fig. 4, paper I). These observations indicate a low or negligible activity of the population of nitrifying bacteria at the start of the experiment, which is reflected by the absence of NO_3^- in the sludge. During the anaerobic digestion of the sludge, the unfavourable conditions for the nitrifying bacteria presumably prevent them from developing and regenerating.

Soil acidity and buffer capacity - relationships to microbial activity (C and N mineralization) (Paper II)

Neither C nor N mineralization (changes in organic N) was significantly affected by different pH levels in irrigation water during the experimental period. Coefficient of determination (R^2) of C mineralization related to pH and plants was explained to 60%, and without outliers it increased to 79.5%. For changes in organic N it was explained to 60% with and 84.8% without outliers respectively. As far as can be interpreted from the experimental data and what is known from the substrate quality, there are two main reasons for the results: 1) Buffering effect of the substrate. Buffering was seen from the leachate and soil pH (Fig. 2, Paper II), although it was shown that pH dropped during the period in some of the treatments. It was indicated that the formation of hydrogen ions was faster in pots with plants where pH in leachate water decreased faster compared to pots without plants. The origin of the additional hydrogen ions in soil solution is most likely from root uptake of NH_4^+ and other cations in exchange for H^+ from the cell metabolism. Hydrogen ions also originate in pots with and without plants from the nitrification process, which was shown to occur after some weeks (Fig. 4, Paper I) but also as a consequence of pyrite oxidation. 2) Heterogeneity in the substrate resulted in high variance between samples.

Plant influence on N mineralization (Paper II)

In the pot trial, the influence of root activity from barley *(Hordeum vulgare)* and red fescue *(Festuca rubra)* on N mineralization (changes of organic N) was studied. According to the literature, the effects on N mineralization are the result of several processes working in opposite directions (Clarholm, 1985; Paustian *et al.,* 1990; Sjödahl Svensson, 1993). This, together with the heterogeneous material, may be the main reason for the absence of significant differences between treatments as an effect of varying root activity.

Synthesis of Paper I and Paper II

Decomposition and N mineralization were measured in both the incubation and pot experiment. The measurements were performed during approximately the same length of experimental period, namely between 13 and 14 weeks. It was therefore possible to make legitimate comparisons between the two experiments.

Before going into comparisons between the two experiments, it is appropriate to point out the likely occurrence of deposition of C-rich exudates and dead roots, which can be estimated to account for up to 40% of the plant's primary C production (Lynch & Whipps, 1990), where 50% (approximately) can be estimated to have been decomposed (T. Kätterer, pers. com.). Quantification of this process is not meaningful due to the high variation in C mineralization. However, a preliminary calculation from the pot experimental data indicated that the C mineralization could have been underestimated due to root-induced C by as much as 25% (or an increase from 18.5 to around 23%). The differences between pots with and without plants on average were still indicated to be large. According to the relatively small influences from what was discussed above, a rough estimation of the 'real' average C losses can be approximated to around 30%. By comparing this value (~30%) with C_{fluxes} from incubated samples (~20%) under optimal conditions (15 ˚C and 24% moisture), it was implied that C mineralization in the pots occurred faster than in incubated vessels. A correction for non-measured respiration during laboratory treatment at room temperature before starting the incubation had to be made. This carbon loss was estimated by temperature response curves from the incubation experiment to around 3% of the initial C content, hence C mineralization from fresh substrate was around 23%. Nevertheless, C mineralization from pots was still higher than from the incubated samples and there is a need to find a valid reason for that. It is possible that there was a higher temperature in the topsoil during sunny days than that adjusted to minimum temperatures (12 ˚C and 15 ˚C) and this is a possible and a reasonable explanation.

Table 2. *Comparisons of mineralized C and N in g kg^{-1} substrate and as a percentage from the incubation- and from the pot experiment, during 92-96 days. The initial C, organic N and mineral N contents were 14.2, 1.59 and 0.35 g kg^{-1} substrate respectively and the C:N ratio ~9. Data from the pot experiment represent the mean values of the three pH treatments.*=moisture content. 1) Changes in organic N, 2) Net N mineralization.*

Experiment	**Treatment**	*C mineralization*		*N mineralization*	
		(g kg^{-1})	(% C)	(g kg^{-1})	(% N)
Incubation	*15˚ C: 14 %**	2.48	17.4	0.24	15.1
	*15˚ C: 24 %**	2.77	19.5	0.23	14.4
Pot	*veg+*	2.63	18.5	0.41 [1)]	25.8
	veg-	5.52	38.8	0.72 [1)]	45.3
	veg+	-	-	0.06 [2)]	4.1
	veg-	-	-	0.06 [2)]	3.7

Changes in organic N roughly followed the amounts of C mineralized in both experiments (Table 2). However, there were tendencies for the C:N ratio to increase in the pot experiment during the experimental period, but also in the incubated samples after estimates of N released from the organic pool during laboratory treatment to be double the decomposed C, or roughly around 6%, which was estimated from the temperature response curves. The impact of changes in C:N ratio was in any case relatively small or less than 1 unit from the initial value of around 9. In the amounts mineralized from the pots, there was an indication that the substrate released relatively more N than C, approximately 26% versus 19% with plants and 45% versus 39% for the bare soil.

The final observation regarding N mineralization was remarkable. The observed amounts of mineral N in the pots (soil and/or plants) were 1/6 and 1/12 of that determined as changes in organic N. A theory for this discrepancy is N losses as gas via denitrification (NO, N_2O or N_2), occurring in water saturated pores or in pores with high respiration rates, or possibly through nitrification (NO and N_2O) if O_2 is available (Davidson, Swank & Perry*,* 1986). Either way, one must hesitate to conclude that such high amounts were lost, which raises the question of whether the changes in organic N values were overestimated. However, the conditions for denitrification in the pots were probably good after 3-4 weeks, since then NO_3^- was available (Fig. 4, Paper I) and this is one important factor for gas formation, along with oxygen limitation and availability of organic C (Tiedje *et al.,* 1988). High denitrification potentials in soils amended with digested sewage sludge with high availability of organic C are not unlikely (Artiola, 1997), and up to 70% of added N was suggested to be denitrified in fermented organic wastes (Volz & Heizel, 1979).

Conclusions

There is a strong influence of temperature and soil moisture on C mineralization and those were the two main factors for decomposition rates in the tailings-sludge mixture. As much as 20% to 30% C may be released from the substrate during 100 days under the climatic conditions assumed to predominate in the field in northern Sweden. At higher temperatures, more C (and N) is mineralized according to the observed temperature function obtained during incubation. Furthermore, there is an indication that plants have a lowering effect on measured N mineralization, primarily due to deposition of C-rich compounds from growing roots into the rhizosphere. High buffering capacity in the sludge is suggested to be the main reason for the lack of observed significant effects from different pH treatments.

The amounts of mineral N found in soil and/or plants at harvest were far less than the total changes in organic N in the tailings-sludge mixture, where considerable amounts were suggested to have left the system via denitrification and to some extent via nitrification.

A future outlook and field applications

How to continue from what we know so far? This type of quantitative study of the decomposition of digested sewage sludge mixed with mine tailings under controlled conditions is far from fully explored. Obviously, we are in a favourable position when planning field research in Aitik in the future. The following are some questions that need to be answered:

1) Is it possible from the observed decomposition and N mineralization during incubation to say that the mineralized amounts are similar in the field? If not, what conditions occurring in the field need to be investigated? One can at least expect that the relative differences in temperature and soil moisture are similar.
2) It is a positive indication, from an environmental point of view, that barley and red fescue reduce N mineralization during growth. Note, however, that the influence of these species has to be more precisely quantified.
3) How can gaseous losses of N be measured and quantified to predict the importance of denitrification (and nitrification) in the field situation in Aitik?

When the sludge is applied to the tailings for the future field study, the degree of decomposition will differ in the organic matter due to differences in storage time and microclimatic conditions (temperature, moisture and oxygen availability). This will have an impact on both respiration rates and N mineralization of the organic matter. Naturally, there is a need to follow up the decomposition of the organic matter during season two, season three and over even longer periods.

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