Extractable and dissolved soil organic nitrogen – A quantitative assessment

Gerard H. Ros a, *, Ellis Hoffland a, Chris van Kessel b, Erwin J.M. Temminghoff a

a Wageningen University, Department of Soil Quality, P.O. Box 47, 6700 AA Wageningen, The Netherlands
b University of California, Department of Plant Sciences, Davis, CA 95616, USA

Article history:
Received 8 August 2008
Received in revised form 7 January 2009
Accepted 12 January 2009
Available online 7 February 2009

Keywords:
Extractable Organic Nitrogen (EON) or Dissolved Organic Nitrogen (DON) pools are often analyzed to predict N mineralisation, N leaching, and to evaluate agricultural (nutrient) management practices. Size and characteristics of both pools, however, are strongly influenced by methodology. Quantifying the influence of methodology can increase the accuracy of soil tests to predict N mineralisation, improve model simulations, and can help to quantify the contribution of the EON and DON pools to soil N cycling. We estimated the relative impact of methodological, management, and environmental factors on EON and DON, using a meta-analysis approach based on 127 studies. Our results indicate that the EON and DON pools are neither similar in size nor controlled by the same factors. The influence of factors controlling EON generally decreased in the order of methodology (ΔI10–2400%), followed by environment (ΔI11–270%) and management (ΔI16–77%). DON concentrations were primarily controlled by management factors: different land use and fertilisation caused a variation of 37–118%. Seasonal variations in DON concentrations were generally smaller than variations in EON, suggesting that high mineralisation and sorption rates buffer DON. The large range in EON as affected by different methodology emphasizes the importance of using appropriate and standardized methods for the determination of EON. The determination of DON can be useful to estimate leaching losses. EON, however, can be used to assess the impact of soil management practices on the turnover rate of labile soil organic matter pools.

1. Introduction

Awareness is growing that Dissolved Organic Nitrogen (DON) plays an important role in ecological processes such as N leaching, mineralisation, and plant uptake (Naºholm et al., 2000; Perakis and Hedin, 2002; Jones et al., 2004; Schimel and Bennett, 2004). It is also well known that a rapid rewetting of dry soils causes an increase in mineralisation and a pulse of available C and N (Fierer and Schimel, 2003). DON originates from plant litter leachates, microbial and root exudates, and hydrolysis of insoluble soil organic matter (Haynes, 2005). Dissolved organic N is defined as the fraction of soil organic nitrogen which is collected in situ using a lysimeter, rhizon or suction cup among other devices, and where no extractant is used. DON is defined as organic N present in dissolved form in soil solution (Murphy et al., 2000). In general, DON concentrations vary between 25 μg L−1 and 10 mg L−1 (Watson et al., 2000; Perakis and Hedin, 2002; Siemens and Kaupenjohann, 2002; Vinther et al., 2006), and account for 0.1–3.0% of soil total N (Haynes, 2005).

By contrast, Soluble Organic N (SON) or Extractable Organic N (EON) is soil N that is extracted from the soil using water, KCl, electro ultrafiltration (EUF), K2SO4, CaCl2, or any other extractant (Murphy et al., 2000). Recently, Xiang et al. (2008) proposed that it would be more appropriate to call soil organic N which is obtained by extraction, EON rather than SON. The majority of results published on extractable organic N, which we used in this meta-analysis, used the term SON. Because the term EON is more appropriate, we use the term EON instead of SON.

The amount of EON in the soil can range from less than 5% of total N by mild salt solution (e.g. CaCl2, diluted acids, etc.) to more than 50% by strong extraction methods such as acid hydrolysis (Stevenson, 1994; Matsumoto and Ae, 2004). Various EON pools are reported to relate with N mineralisation (Appel and Mengel, 1998; Mulvaney et al., 2001; Sharifi et al., 2007), land use changes, and agricultural management practices (Haynes, 2005). Typically, EON is extracted from field moist or dried soils by shaking with water or a salt solution at a high soil-solution ratio for short periods of time, followed by centrifugation or filtering to separate the solution phase from the solid phase (Jones and Willet, 2006).

Conceptually, DON can be considered as a sub-pool of potentially EON that exists as a part of soil organic matter N. Tipping (1998) postulated that the EON pool “is a part of the soil solids and...
able to pass into solution under realistic soil conditions”. We consider EON as the sum of DON plus extra organic compounds that solubilise during extraction, originating from soil biomass and solid organic N (Fig. 1). The DON and EON pools are controlled and replenished by organic matter inputs (litter, manure), exudates, soil organic N, and influenced by adsorption–desorption and by microbial activity (Kalbitz et al., 2000). In soil, DON and EON pools are assumed to be in equilibrium (Qualls, 2000; Gjettermann et al., 2008). Size and characteristics of EON, however, depend strongly on how the EON pool is extracted (Stevenson, 1994; Matsumoto and Ae, 2004). Therefore, it is important to know the impact of methodology on the quantity and quality of EON and DON. An understanding of the impact of methodology on the size of the pools can lead to an increase in the accuracy of soil tests used to predict N mineralisation, to improve model simulations, and to quantify the contribution of DON to N leaching and plant N uptake.

Both DON and EON are mainly controlled by similar processes (Kalbitz et al., 2000; Qualls, 2000): mineralisation and immobilization, adsorption and desorption, and precipitation and solubilisation. The release of DON rather than EON, however, is considered as a key controlling mechanism of terrestrial N cycling (Schimel and Bennett, 2004). We hypothesized that environmental parameters have a smaller impact on EON concentrations than on DON because of differences in size between the two pools (Fig. 1) and differences in chemical characteristics. Foremost, the extra solubilised compounds in EON are considered as organic N that potentially dissolves in time, having a lower turnover rate than DON (Tipping, 1998; Kalbitz et al., 2000; Zsolnay, 2003). Consequently, the concentration of DON is likely more affected by changes in microbial activity than EON. The turnover rate of DON is higher than that of EON, because EON is partly physically protected (Zsolnay, 2003). DON is also enriched with labile hydrophilic compounds due to the selective sorption of recalcitrant, aromatic, and hydrophobic compounds from the soil solution (Guggenberger and Kaiser, 2003; Kalbitz et al., 2003). These hydrophilic compounds include small carboxylic acids, proteins, sugars, free amino acids and amino sugars (Qualls and Haines, 1991). However, these compounds comprise not only a range of moderately transformed plant-derived polysaccharides as well as microbial metabolites (Qualls and Haines, 1991), but also compounds of cell lysis (Christ and David, 1994). Drying soils before extraction, enhances the extraction of labile hydrophilic compounds (Kaiser et al., 2001), probably increasing the turnover rate of EON. In this situation, the impact of environmental parameters may have a bigger impact on EON than on DON, but it reflects a change in biomass rather than a change in an active soluble/dissolved pool of organic N. Changes in the size of the EON pool are also less apparent than a change in DON, because the size of the EON pool is generally larger than the active DON pool. Lastly, conditions that change the dynamics of the soil solution have a stronger effect on DON than on EON as part of the EON pool is adsorbed to the soil matrix. Conditions that change the dynamics of the soil solution include dilution after rainfall, transport, high ion concentrations after fertilisation, and inputs from the rhizosphere. In contrary to our hypothesis, it can be argued that following changes in environmental conditions or management practices, high mineralisation (Jones et al., 2004, 2005) and sorption rates (Kaiser and Zech, 1998) will reduce variations in the size of the DON pool. Consequently, measuring the pool size only may underestimate the size of the active DON pool. At present, however, it is not fully known whether EON and DON do react differently following a change in environment conditions or management practices.

As most studies on EON are focussed on statistical relationships with soil parameters (e.g. predicting N mineralisation) or human activities (e.g. soil management), a mechanistic understanding of this pool, and its interaction with DON, remains scant. This scant understanding is further hampered by methodological differences among studies (McDowell, 2003; Zsolnay, 2003). Variations in DON and EON pool size have been observed following a change in land use (Willett et al., 2004; Christou et al., 2005), seasonal variation (Leinweber et al., 1995; Jensen et al., 1997; Vinther et al., 2006; Weintraub and Schimel, 2005), drought and freezing (Schimel et al., 2007), drying and wetting cycles (Fierer and Schimel, 2002; Miller et al., 2005) and changes in management practices (Chantigny, 2006).
Differences in soil characteristics also lead to differences in the size of the DON and EON pools (Kalbitz et al., 2000). The observed differences in the size of the EON pool following a change in land use, season, or management practices may be related to differences in the methodology used to determine the EON pool. However, studies on the size of DON and EON pools that separate the influence of methodology from environmental factors and management practices are scant.

We quantified the mean influence of methodology, environment and nutrient management on DON and EON levels, using the meta-analysis approach. This statistical technique reckons with methodological differences between studies and integrates independent data quantitatively (Gurevitch and Hedges, 1999, 2001). We tested the hypothesis that DON concentrations are more prone to changes in environment and nutrient management than EON concentrations. More specifically, we addressed the following objectives: 1) to determine how much of the variation in DON and EON concentrations is related to methodology versus management practices and environmental factors, and 2) to assess whether methodology, management practices and environmental factors have a similar impact on DON and EON.

2. Data analysis

A meta-analysis can be used to estimate the average response of how DON and EON concentrations vary across a large number of studies due to a change in biophysical conditions or methodology, to test whether the change in biophysical conditions or methodology is significantly different from zero, and to examine the cause and effect of differences in DON and EON concentrations induced by changes in biophysical conditions or methodology. Data in a meta-analysis generally take the form of standardized metrics of an effect size and their associated sampling variances (Gurevitch and Hedges, 2001). We calculated the effect size in each experiment as the natural log of the response ratio ($R$, relative difference between 2 groups). The response ratio was calculated by dividing the mean of one group by the mean of a control group (Hedges et al., 1999; Rosenberg et al., 2000). For example, the influence of land use on DON levels was determined by calculating a relative difference between DON levels in arable, grassland and forest soils, using arable soils as control group. The mean difference between two groups among the analyzed studies was calculated as described in Gurevitch and Hedges (2001).

Mean DON or EON levels of experimental and control groups with their standard deviations (SDs) and replicates ($n$), from a large number of studies were collected. A total of 200 studies published between 1980 and 2008 were identified, of which 127 studies included quantitative data for the control and treatment groups (reported in the Further reading). Data were subdivided in various subgroups related to the factors that could affect the concentration of DON or EON (Table 1). To obtain sufficient data which would allow us to use the meta-analysis approach, studies which did not report SD or SE values were included in the analysis by using an arbitrary SD value based on a coefficient of variation (CV) of 1.5 times the average CV in the other studies (Alberton et al., 2005). Only studies which showed replication of the treatments were included. Error bars not identified were assumed to represent SE. If several values of the number of replicates were given, the lowest value was taken.

The mean difference between two groups is significantly different from 0 if both the upper and lower confidence limits were positive or negative. When the pooled within-class variance ($\sigma^2$ pooled) was $>0$, a random effect model was used, whereas a fixed effect model was used when that quantity was equal to or $<0$. Means of response variables of different subgroups and differences between DON and EON were tested for significant differences based on the model heterogeneity test ($Q$-test), which is tested against a chi-square distribution with $n – 1$ degrees of freedom as implemented in MetaWin ($P < 0.05$).

When insufficient quantitative data were available for the meta-analysis, we reviewed the influence of methodology on concentrations of DON and EON using results from individual studies. Methodological changes in the quality of DON and EON were assessed using their influence on the contribution of labile compounds to the total amount of DON and EON. Relevant observations from research on Dissolved Organic C or Extractable Organic C pools were also included in this analysis.

3. Results

3.1. Influence of methodology on DON and EON

Most of the variations in extraction method significantly affected levels of EON (Table 2, Fig. 2). The EON levels increased with increasing drying temperature prior to extraction, extraction temperature ($T$), molarity of salt solutions, and soil-solution ratios. Centrifugation, dilution, and filtration decreased the amount of EON. The mean difference between dried and field-moist soil, hot and cold extraction temperatures, and among several weak salt solutions was quantified (Fig. 2). Foremost, drying soil samples at 20 °C prior to extraction caused an average increase in EON of 245%. The increase was related to the drying temperature ($p < 0.001$); soils dried at 20, 40 or 105 °C showed an average increase of EON of 245, 400, and 2400%, respectively. Second, extraction of soil samples with water resulted in significantly lower contents of EON than when 0.01 M CaCl$_2$ or 1–2 M KCl was used (Fig. 2). Comparing

| Table 1 |
| Factors affecting DON or EON concentrations analyzed by meta-analysis. |

<table>
<thead>
<tr>
<th>Controlling factors</th>
<th>Subgroups</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil drying</td>
<td>Soils that are analyzed field moist, or dried (dried at 20, 40, or 105 °C)</td>
<td>Field-moist soils</td>
</tr>
<tr>
<td>Extraction T</td>
<td>Soils extracted at a temperature above 80 °C or at room temperature</td>
<td>Soils extracted at room temperature</td>
</tr>
<tr>
<td>Salt solution</td>
<td>Soils extracted with water, K$_2$SO$_4$, CaCl$_2$, Electro Ultra Filtration, or KCl</td>
<td>Soils extracted with CaCl$_2$ or KCl extract</td>
</tr>
<tr>
<td>Land use</td>
<td>Arable, grassland, and forest soils</td>
<td>Arable soils</td>
</tr>
<tr>
<td>Fertilizer application</td>
<td>N-fertilizer and no N-fertilizer application</td>
<td>No N-fertilizer application</td>
</tr>
<tr>
<td>Seasonality</td>
<td>Soils sampled in spring, summer, autumn or winter</td>
<td>Soils sampled in winter</td>
</tr>
<tr>
<td></td>
<td>Soils sampled in autumn/winter, and soils sampled in spring/summer</td>
<td>Soils sampled in autumn and winter</td>
</tr>
<tr>
<td>Soil depth</td>
<td>Topsoil ($&lt;30$ cm; A-horizon) and subsoil ($&gt;30$ cm; B/C horizon)$^a$</td>
<td>Topsoil</td>
</tr>
<tr>
<td>Soil pH</td>
<td>Soils with a pH $&gt;6$ or $&lt;6$</td>
<td>Soils with a pH $&lt;6$</td>
</tr>
<tr>
<td>Soil texture</td>
<td>Clay, silt and sandy soils$^b$</td>
<td>Sandy soils</td>
</tr>
<tr>
<td>Total soil N content</td>
<td>Soils with total N content $&lt;2$ g kg$^{-1}$, between 2 and 4 g kg$^{-1}$, or $&gt;4$ g kg$^{-1}$</td>
<td>Soils with total N content $&lt;2$ g kg$^{-1}$</td>
</tr>
</tbody>
</table>

$^a$ Litter horizons of forest soils are excluded from the analysis.

$^b$ The sandy group included texture class sand and all sandy soils; the silt group included silt and all silty soils; the clay group included all soils with at least 50% clay. Observations on clay loam soils were used in both the silt and clay groups whereas loam soils were used in both the sand and the silt groups.
other extraction methods, electro ultrafiltration (EUF) and NaHCO₃ extraction resulted in significantly higher EON levels than when CaCl₂ was used, whereas K₂SO₄ extracted lower amounts of EON than CaCl₂. Finally, hot extractions (80–100 °C) resulted in significantly higher amounts of EON than cold extractions (20 °C) with an average increase of 147% (Fig. 2). Looking at the individual extraction methods, T showed a significant effect with an average increase of 95% when CaCl₂ was used, whereas K₂SO₄ extracted lower amounts of EON.

Although the influence of methodology on the characteristics of EON was often unclear, its stability decreased with an increase in the drying T, and an increase in the soil-solution ratios (Table 2).

### 3.2. Influence of management practices on DON and EON

Levels of DON and EON were affected by land use (Fig. 3). Compared to arable soils, average DON concentrations were lower (−35%) in forest soils and grassland soils (−64%). In contrast, average EON content was higher in grassland soils (+81%) than in arable soils. Compared to arable soils, forest and grassland soils differed significantly in DON and EON levels (p < 0.01).

A maximum increase of 118% was found in EON levels following the application of inorganic and organic fertilizers (Fig. 3). DON increased with 57% following the application of inorganic N-fertilizers whereas EON increased with 17%. Incorporation of crop residues or manure increased EON concentrations with 22% and 70%, respectively.

### 3.3. Influence of environmental factors on DON and EON

DON concentration measured in spring/summer did not differ significantly from autumn/winter measurements (Fig. 4). Average EON concentrations, however, were significantly higher in spring/summer (+56%) compared to the autumn/winter period. When individual seasons were compared, significant differences in EON concentrations between the winter and autumn (+110%), and between winter and summer (+268%) were found.

Levels of DON and EON decreased with depth, with an average difference of 36–49% between the topsoil and subsoil. Decreases in DON and EON concentrations by depth were almost similar (36 and 49%; p = 0.043).

Whereas an increase in pH (pH > 6) led to significantly lower EON concentrations (−22%), it caused DON concentrations to become highly variable (Fig. 4). Overall, the response of DON and EON to soil pH was significantly different (p = 0.012). These results agreed with the effect on liming, which showed a tendency (p > 0.05) to decrease EON and increase DON concentrations (Fig. 3).

The concentration of EON was positively related to total soil N (Fig. 4). Compared to soils with an N content of <2 g kg⁻¹ the EON levels increased by 240% for soils when the total N content was >4 g kg⁻¹. For soils with a total soil N content between 2 and 4 g kg⁻¹ the EON concentration increased by 61%.

The EON content in non-sandy soils was 40–50% higher than in sandy soils. EON content did not differ significantly between silt and clay soils (p = 0.698). Due to lack of sufficient data, the influence of total N and texture on DON concentrations could not be quantified.

### 4. Discussion

#### 4.1. Influence of methodology on EON contents

The most important factor that causes the soil EON content to change is the method which is used to extract EON. Depending on

---

**Table 2**

Summary of factors controlling levels and characteristics of EON.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Variation</th>
<th>Quantity*</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage temperature soil (°C)</td>
<td>−20 to 70</td>
<td>−/+</td>
<td>0/+</td>
</tr>
<tr>
<td>Drying temperature soil (°C)</td>
<td>20–105</td>
<td>++</td>
<td>−/+</td>
</tr>
<tr>
<td>Drying soils (−)</td>
<td>yes/no</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Sieving soil (mm)</td>
<td>&lt;1 to 10</td>
<td>0/−</td>
<td>?</td>
</tr>
<tr>
<td>pH of extractant (−)</td>
<td>weak–strong salts</td>
<td>−/+</td>
<td>−/+</td>
</tr>
<tr>
<td>Soil-solution ratio (g L⁻¹)</td>
<td>25–500</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Extraction temperature (°C)</td>
<td>5–120</td>
<td>++</td>
<td>−/+</td>
</tr>
<tr>
<td>Extraction time (min)</td>
<td>5–1440</td>
<td>++</td>
<td>?</td>
</tr>
<tr>
<td>Dilution (−)</td>
<td>1–10</td>
<td>0/−</td>
<td>+</td>
</tr>
<tr>
<td>Filtration (µm)</td>
<td>0.1–0.45</td>
<td>–0/+</td>
<td>0/+</td>
</tr>
<tr>
<td>Centrifugation (−)</td>
<td>yes–no</td>
<td>−/+</td>
<td>0</td>
</tr>
<tr>
<td>Centrifugation time (min)</td>
<td>5–60</td>
<td>−/+</td>
<td>?</td>
</tr>
<tr>
<td>Centrifugation force (g)</td>
<td>5,000–20,000</td>
<td>−</td>
<td>?</td>
</tr>
<tr>
<td>Storage soil solution (°C)</td>
<td>−20 to 20</td>
<td>−/+</td>
<td>0/+</td>
</tr>
</tbody>
</table>

* ++ strong positive influence; + positive influence; 0 no clear trend; − negative influence; ? unknown influence.

---

**Fig. 2.** Average change in % of the concentration of EON due to soil drying (dried soils compared to field-moist soils), extraction solution temperature (T > 80 °C compared to T = 20 °C), and salt solution (CaCl₂ and KCl are used as reference). Error bars denote 95% confidence interval. * N = Number of observations.
which method is used, it could change the EON content by 2400% (Fig. 2). The significant increase in EON due to an increase in drying T prior to analysis (Fig. 2; Table 2) likely originates from lysis of microbial biomass following desiccation and from disrupted organic-mineral associations and subsequent release of organic compounds (Haynes et al., 1991; Haynes, 2005; Appel et al., 1996; Appel and Mengel, 1998). Similarly, an increase in extraction T, the molarity of salt solutions, and soil-solution ratios increases EON contents (Matsumoto and Ae, 2004; Kaiser et al., 2001; Fest et al., 2008) due to their effect on sorption equilibria. Centrifugation, dilution, and filtration however, decrease the amount of EON by flocculation or adsorption, depending on the filter size used, centrifugal force, pH and the initial characteristics of the particulate organic matter (POM) fractions (Table 2; You et al., 1999; Rees and Parker, 2005). POM fractions consist of organic particles that are not bound to mineral particles (Haynes, 2005; Gregorich et al., 2006). POM is an intermediate between plant residues and soil organic matter (Gregorich et al., 2006) and interacts with DOM. For example, Zsolnay (2003) illustrated how DOM could be transformed into POM during filtration because of changes in its tertiary structure. Unfortunately, the size of the database was too limited to better quantify the influence of all methodological differences on EON.

Methodological differences are also known to influence the size of the biologically active part of the EON pool (~stability; Table 2). The increase in EON contents following drying the soil prior to extraction mostly consists of easy degradable compounds (Appel and Mengel, 1990, 1993; Nunan et al., 2001). Drying soils
prior to extraction is therefore likely to result in an increase in the biologically active EON pool. Decreasing stability of EON is also related to increasing soil-solution ratios (Kaiser et al., 2001), filtration (You et al., 1999; Rees and Parker, 2005) and the use of specific salt solutions (Rennert et al., 2007). Calcium, for example, is able to reduce the solubility of high molecular weight compounds (Römken and Dolfing, 1998; Reentsma et al., 1999; Rennert et al., 2007). Consequently, the relative contribution of smaller compounds will increase. High extraction temperatures (\(>80^\circ C\)) and use of stronger salt solutions however, increase the solubilisation of recalcitrant compounds and are subsequently related to an increase in stability of EON (Table 2; Michrina et al., 1982; Matsumoto and Ae, 2004). Because the biochemical characteristics of EON affect its contribution to N mineralisation and sorption, qualitative information about the EON pool remains important.

Extraction conditions and prior sample treatment strongly affect whether more or less microbial N, physically protected, chemically adsorbed or solid organic N is solubilised and extracted. This will definitely impact the role of the analyzed EON in soil N cycling, and most likely also the value of EON as an indicator for bioavailable N. But how and to what extent, is not known, yet. Accounting for this impact is necessary in increasing our understanding of the role of EON in soil N cycling. The strong impact of methodology also emphasizes the importance of clearly describing the methodology used to obtain EON. There is a strong need of consistency when the methodology is been developed for the relationship between extractable soil N and plant N uptake (Wang et al., 2001; Griffin, 2000). There is a strong need of consistency when the methodology is been developed for the relationship between extractable soil N and plant N uptake (Wang et al., 2001; Griffin, 2000).

Methodology is known to affect the chemistry of the soil solution (Ludwig et al., 1999; Geibe et al., 2006; Wetherrmiller et al., 2007), and is therefore also likely to affect DON concentrations. For example, the amount and characteristics of DOM vary among pore size classes (Zsolnay, 2003) and consequently, DON concentrations are affected by the centrifugal force or amount of suction applied to collect the soil solution. In addition, specific sorption and contamination from solvents and flexibilisers may induce potential artefacts (Wetherrmiller et al., 2007). Until now, there is still a need to perform a quantitative and qualitative comparison of common devices used to collect DON.

### 4.2. Difference in response of DON and EON to management and environment

In general, changes in land use and nutrient management caused a different response of the size of the DON and EON pools (Fig. 3). Likewise, changes in the DON and EON concentrations induced by season, soil depth or pH were different (Fig. 4). As both pools are mainly controlled by immobilization, mineralisation, sorption and desorption (Kalbitz et al., 2000; Qualls, 2000), a different response implies that the relative impact of these processes differ for both pools.

#### 4.2.1. Impact of land use and fertilisation on DON and EON

Larger variations in DON compared to EON were only observed as a response to inorganic N fertilisation and when forest soil was compared with arable soils. Following the application of inorganic N-fertilizer it is known that the concentration of DON increases, as the application of inorganic N-fertilizer solubilises soil organic N because of its pH effect (Chantigny, 2003). The addition of inorganic N-fertilizer changes the microbial community structure, and increases the hydrophilic character of DON due to an increased production of N-rich fractions or a decrease in consumption (McDowell et al., 2004). Although the variation in the absolute concentration of EON is larger than that for DON (data not shown), the relative increase in EON content following the application of fertilizer-N is only 10% whereas for DON the increase was 50% (Fig. 3). DON concentrations were lower in forest soils than that in arable soils, whereas concentrations of EON tend to be higher in forest soils. Dissolved organic C and extractable organic C levels were also significantly higher in forest than arable soils (data not shown). Higher levels of C often contribute to a lower biodegradability of organic compounds: forest litter contains higher lignin content and C to N ratio than agricultural crop residues (Chantigny, 2003). Lower DON levels in forest and grassland soils, therefore, could be the result of higher immobilization rates or sorption: more N is needed for decomposition of C rich compounds and these compounds show also a stronger sorption affinity (Guggenberger and Kaiser, 2003).

High release of root exudates in grassland soils is likely to increase the rate of immobilization of DON compared to arable soils, leading to a decrease in the concentration of DON (Khalid et al., 2007). This agrees with our observation that the average DOC concentrations were higher in grassland soils than arable soils (data not shown). In contrast to DON, the concentrations of EON were higher in grassland soils than in arable soils which is likely related to their higher organic matter and biomass content (Haynes, 2000). Because most measurements of EON are performed on dried soils, higher EON contents in grassland soils partly reflect a higher contribution of lysed microbial cells.

#### 4.2.2. Seasonality

The most important factors controlling DON leaching losses are likely related to water fluxes through the soil profile and without significant precipitation or irrigation, leaching will not occur (Van Kessel et al., 2008). Rewetting and drying of soils which are common occurrences in arid, semi-arid, or Mediterranean-type environments, have short term but pronounced effects on the content of soluble organic compounds and N mineralisation in surface and subsurface soil (Franzluebbers, 1999; Fierer and Schmidt, 2002; Xiang et al., 2008). Increasing the frequency of wetting and drying and adding Adenostoma litter had no effect of CO2 release but significantly increased DON concentrations (Miller et al., 2005). Although DON concentrations are related to the contact time between the soil and the soil solution (McDowell and Wood, 1984; Michalzik and Matzner, 1999), suggesting seasonal variation in DON concentrations, we observed no significant influence of different seasons on DON concentrations (Fig. 4). In contrast, EON concentrations were significantly different between summer and winter periods, and during autumn and winter periods. Changes in seasons lead to differences in temperature and moisture which are important factors controlling microbial activity and its adaptation (Schmidt et al., 2007). An absence of a significant change in the DON concentration would suggest that microbial assimilation and/or the rate of mineralisation caused the concentrations of DON to remain low. The DON pool is often characterised to be highly dynamic with a high turnover rate (<4 h) (Kaiser and Zech, 1998; Jones et al., 2005; Van Hees et al., 2005). It is possible that differences in the size of the DON pool following a change in season cannot be detected when measured at weekly or monthly intervals. As abiotic (sorption equilibria) and biotic (microbial uptake and release) processes control the concentration of DON, the most pronounced change in the DON concentration is likely to occur in the surface layer and not in the subsoil. As seasonal measurements of DON, however, are often determined below the cultivated layer which show lower DON concentrations (Van Kessel et al., 2009), seasonal variations in DON concentrations will be smaller.
4.2.3. Soil characteristics

Because the majority of the organic matter input occurs in the plough layer, the topsoil layer shows the highest soil organic matter content and microbial activity which will decrease with depth. Likewise, the concentrations of EON and DON are the highest in the topsoil and decrease by depth (Fig. 4). Although the difference between DON and EON due to depth is significant (p = 0.04), the different response to depth is small (about 10%). The decrease in the EON concentration as affected by an increase in soil pH (Figs. 3 and 4) may be explained by its increasing solubility (Andersson et al., 1994), increased microbial activity and consumption of soluble molecules (Karluk, 1995), or an increased sorption by cation bridging due to high Calcium concentrations (Römkes and Dolfing, 1998). Although decreases in the contents of EON are likely to cause higher DON concentrations, the dataset was not large enough to be able to draw a firm conclusion. The influence of soil pH on DON is therefore less clear. If similar processes affect DOC and DON concentrations, the effect of pH would be small within the normal pH range as they occur in agricultural soils (Kalbitz et al., 2000).

Levels of DON are likely to increase with increasing total soil N and a decrease in clay content (Kalbitz et al., 2000), but the meta-analysis approach could not be used to quantify this increase.

4.3. Concluding remarks

Although a mechanistic understanding of the functions of EON remains limited, its usefulness as indicator of how agricultural management strategies affect soil N dynamics has been proven. Significant variation in EON as affected by total soil N content, soil pH, fertilisation, and land use supports this observation. Because of substantial seasonal variability, it emphasizes the need that sampling be carried out at the same time each year in order to make comparison possible. Otherwise, temporal variation may obscure differences due to changes in management practices. Even more important is the impact that methodology can have on the concentration of EON. Accounting for this impact is necessary to make progress in increasing our mechanistic understanding of the role of EON in soil N cycling.

Compared to DON, the content of EON was more prone to changes in seasons, soil depth, pH, and cropping systems. These findings invalidate our hypothesis that variations in the concentrations of DON are more pronounced than variations in EON contents. They support the suggestion that the flux of organic compounds through both the DON and the EON pools is affected by changes in biophysical–environmental conditions and management practices. When the system is not at steady state (e.g. in response to N-fertilizer), changes in the N flux through the DON and EON pools will cause a change in their characteristics, because both sorption and mineralisation are selective for specific compounds. Qualitative information of both the DON and the EON pools, therefore, will also be useful to understand their role in the soil N cycle.

Acknowledgements

G.H.R. was financially supported by the Technology Foundation STW and Bbag. We thank Dr. J.P. Schimel and three anonymous reviewers for their helpful comments on an earlier version of this paper. We thank also the financial support of the C.T. de Witt Graduate School at Wageningen University, the Netherlands (C.V.K.).

References


Further reading: studies used for meta-analysis

organic nitrogen in long-term experiments with maize. Plant, Soil and Environ-
ment 49, 560–564.
Cookson, W.R., Müller, C., Bruce, L.G., Clark, L., Milton, N., Smirk, M.N., Murphy, D.V., Osmond, M., Stockdale, E.A., Hurst, P.R., 2006b. The influence of season, agricul-


Steinberger, B., Kjønaas, O.J., Chemical characterization of soil chemistry during four years of NH4NO3 addition to a forested catchment at Gardsjön, Sweden. Forest Ecology and Management 101, 215–226.


