Increasing trends of dissolved organic nitrogen (DON) in temperate forests under recovery from acidification in Flanders, Belgium

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HIGHLIGHTS
• DON concentrations increased under acidification recovery.
• DON fluxes showed similar increasing trends.
• DOC:DON peaked after drying and rewetting.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 8 February 2016
Accepted 8 February 2016
Available online xxxx

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Keywords:
Dissolved organic nitrogen
Dissolved organic matter
Temperate forests
Acidification
Temporal trends
ICP Forests

ABSTRACT

We evaluated trends (2005–2013) and patterns of dissolved organic nitrogen (DON) and its ratio with dissolved organic carbon (DOC), DOC:DON in atmospheric deposition and soil solution of five Level II plots of the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) in Flanders, Northern Belgium. The primary aim was to confirm positive postulated trends in DON levels and DOC:DON under on-going recovery from acidification. The DON concentrations (0.95–1.41 mg L⁻¹) and fluxes (5.6–8.3 kg ha⁻¹ y⁻¹) in throughfall were about twice as high compared to precipitation in the open field (0.40–0.48 mg L⁻¹, 3.0–3.9 kg ha⁻¹ y⁻¹). Annual soil profile leaching losses of DON varied between 1.2 and 3.7 kg ha⁻¹ y⁻¹. The highest soil DON concentrations and fluxes were observed beneath the O horizon (1.84–2.36 mg L⁻¹, 10.1–12.3 kg ha⁻¹ y⁻¹). Soil solution concentrations and fluxes of DON showed significant increasing trends. Temporally soil solution DOC:DON rose following an exceptionally long spring drought in 2007, suggesting an effect of drying and rewetting on DOM composition. Further research is needed to test the dependence of DON and DOC:DON on factors such as latitude, forest cover, length of the growing season, hydrology and topography. Nonetheless, even with considerable variation in soil type, level of base saturation, and soil texture in the five included ICP Forests Level II plots, all data revealed a proportionally larger positive response of DON flux than DOC to recovery from acidification.

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Abbreviations: BD, deposition through precipitation in the open field; TF, deposition below canopy (throughfall + stemflow).
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http://dx.doi.org/10.1016/j.scitotenv.2016.02.060
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1. Introduction

Atmospheric deposition of inorganic nitrogen (N) and sulphate (SO$_4^{2-}$) caused N saturation and a fast acidification of temperate forest soils and waters in large parts of Europe and the US during the second half of the 20th century (Aber et al., 1989; van Breemen et al., 1984). This was found to have an impact on dissolved organic matter (DOM) cycling, often resulting in higher concentrations and leaching of DOM (Kalbitz et al., 2000; McDowell et al., 2004; Pregitzer et al., 2004). National policies and international cooperation to abate acidifying emissions implemented since the late 1970’s, like the Convention on Long-range Transboundary Air Pollution (CLRTAP), resulted in a significant lowering of acidifying deposition. In European forests, Waldner et al. (2014) observed an overall decrease of SO$_4^{2-}$ depositions by 6% per year between 2000 and 2010.

Long-term positive trends of surface water dissolved organic carbon (DOC) concentration across Europe, Canada and the US occurred simultaneously (de Wit et al., 2007; Monteith et al., 2007). These positive DOM trends in stream waters and soil solutions of highly acidified forests were mainly linked to the declining SO$_4^{2-}$ depositions and subsequent initial chemical recovery of the soil solution (de Wit et al., 2007; Monteith et al., 2007; Oulehle et al., 2011). As postulated by Monteith et al. (2007), decreasing soil solution SO$_4^{2-}$ concentrations will lower soil solution ionic strength, leading to an increased solubility of DOM. In addition, the resulting higher pH would increase DOM solubility by lowering soil solution concentrations of ionic aluminum (Al$^{3+}$). For temperate forests, however, negative or indifferent multi-year trends in leachate DOC concentration were seen as well (Aksesson et al., 2013; Borken et al., 2011; Löfgren and Zetterberg, 2011; Monteith et al., 2007; Oulehle et al., 2011; Vanguelova et al., 2010; Verstraeten et al., 2014). Contrary to DOC, still little long-term data exists on DON concentrations and vertical fluxes in temperate forests under recovery from acidification. Mainly so, because monitoring of DON was only recently introduced in forest monitoring networks. Although the concentrations of DON in throughfall water and soil solution are correlated with the concentrations of DOC, since N-containing organic building blocks (proteinaceous or heterocyclic-N) are also comprised in the DOC and DON of a spruce forest showing acidification effects (Michalzik et al., 2001; Qualls and Haines, 1991; Wu et al., 2010), it is not clear to what extent DON concentrations follow trends in DOC. Oulehle et al. (2011) observed parallel trends in soil solution DOC and DON of a spruce forest showing acidification recovery in the Czech Republic. Vanguelova et al. (2010) found that a positive trend of DON was not always coupled to a positive trend of DON at 10 ICP Forests Level II sites in the UK. It should be noted though, that both studies were shorter than nine years identified as minimum for distinguishing clear trends in DOM (Waldner et al., 2014).

When investigating deposition effects on DON, also account has to be taken of evolutions in inorganic N deposition, as it also impacts DOM cycling (Brookshire et al., 2007; Campbell et al., 2000; Currie et al., 1996; McDowell et al., 2004; Pregitzer et al., 2004). In European forests, inorganic N depositions decreased by 2% per year between 2000 and 2010, but only in certain regions, while other regions showed stable or increasing N depositions (Waldner et al., 2014). It is still unclear, however, if an increase in the DOC:DON ratio could be expected under recovery from N deposition.

Finally, increasing trends of DON have also been linked to climate warming (Vanguelova et al., 2010), with often observed positive correlation between the concentrations of DOC and DON in throughfall and air temperature (Sleutel et al., 2009; Solinger et al., 2001; Wu et al., 2010). Increasing throughfall inputs of DON could explain positive DON trends in soil solution (Vanguelova et al., 2010). Throughfall inputs are an important source for DOM in soil solution, and contribute relatively more to DON than to DOC in soil solution (Guggenberger and Zech, 1994; Michalzik et al., 2001; Sleutel et al., 2009; Solinger et al., 2001). Higher air temperatures could increase the activity of herbivorous insects in the canopy, which could lead to higher throughfall fluxes of DOM (Michalzik and Stadler, 2005; Pitman et al., 2010). Higher soil temperatures also stimulate decomposition and mineralization processes, which could increase DOC and DON leaching from the forest floor (Andersson et al., 2000; Michalzik et al., 2001).

The complex interplay of biological and physico-chemical factors that influence DON and DOC cycling, make it difficult to predict the net effects of shifts in climate or deposition onto vertical DOM balances. Long-term data from intensive monitoring plots accounting for all these potential environmental drivers, are crucial to forward our understanding of changing DOC and DON fluxes in forests. We monitored the concentrations and fluxes of DON and DOC in the deposition and soil solution of five ICP Forests intensive monitoring plots (Level II) in Flanders (Belgium) and examined the data for unique long-term trends (2005–2013) and patterns of DON and DOC:DON. Positive DOC trends were recently observed at the five plots following a sharp decline in atmospheric SO$_4^{2-}$ deposition (Verstraeten et al., 2012, 2014), and we hypothesize that concentrations and fluxes of DON in the deposition and soil solution followed the DOC trends. Since N-poor DOM compounds generally have a lower reactivity with Fe and Al hydroxides, a pH control on DON solubility is probably smaller than for DOC. Hence, we hypothesized that recovery from acidification disproportionally favors DOC dissolution compared to DON and the DOC:DON ratio would increase over time. Under field conditions, however, pH-dependent processes like microbial degradation and plant growth also govern DOC and DON levels, complicating the purely abiotic pH-DOM-solubility relation. The relevance of abiotic and biotic DOM transformations is furthermore very much depth-distributed, e.g. depending on Fe and Al content of soil horizons. A second objective thus was to firstly detect and interpret change in DOC:DON ratio in the five ICP Forests Level II plots by means of an extensive 9-year fortnightly dataset.

2. Materials and methods

2.1. Study area

Flanders (Northern Belgium) has a moderate Atlantic climate with a mean annual precipitation of 852 mm and mean temperature of 10.5°C (long-term averages for 1981–2010 for the meteorological station of Uccle, www.meteo.be). Five plots of the ICP Forests intensive monitoring network (Level II) in Flanders were included in this study. These plots were installed in 1987 (circular plots, with an area of 0.25 ha each). Relevant site and soil characteristics are listed in Tables 1 and 2.

Two plots are located in coniferous forest: Pinus sylvestris L. in Brasschaat (BRA) and Pinus nigra ssp. laricio var. Corsicana Loud. in Ravels (RAV). Both stands are situated in the northern Campine ecoregion on a sandy soil (Arenosol) with a C:N ratio of 30–35 in the organic layer (mor humus). The soil profile in RAV is well-drained, while in BRA the infiltration of water is locally slowed down by clay lenses at 50–125 cm depth. Three other plots are installed in deciduous forest: Fagus sylvatica L. in Wijndendale (WIJ) and Hoelstraart (HOE) and a mixture of Quercus robur L. and F. sylvatica L. in Controde (GON). WIJ is located in the western part of sandy Flanders, on a sandy loam soil (Umbrisol), HOE is located in the Loess belt of Flanders, on a silt to silt loam soil (Alisol) with deep groundwater table (> 30 m). GON is located on the slopes of a brooklet valley in the Dender-Klein Brabant ecoregion, on a silt loam to loam soil on top of a heavy clay subsoil (Planosol). The deciduous stands have a C:N ratio of 22–25 in the organic layer (mor humus).

2.2. Sample collection and measurements

Samples of deposition and soil solution were collected fortnightly from January 2005 till December 2013, according to the guidelines of the ICP Forests manual, part XI and XIV (ICP Forests, 2010). Precipitation was sampled with four bulk collectors, located in the open field nearby each plot. Bulk collectors consisted of a polyethylene funnel (14 cm Ø)
placed at 1 m height, which was connected to a subterranean 2 L polyethylene bottle. A nylon mesh (1 mm² mesh size) was placed in the funnel to avoid contamination by large particles. Throughfall was sampled in each plot with ten systematically distributed bulk collectors of the same type as open field collectors. At every sampling event, the volume collected in each bulk collector was determined and samples were bulked to one sample. Stemflow was determined at every sampling event. Subsamples were taken from all full containers, with subsample volumes weighted to tree diameters, and bulked to one sample. Stemflow volumes, obtained from the individual trees, were upscaled to plot level using information of basal area.

Soil solution draining from the O horizon (forest floor) was sampled with four (RAV, BRA, GON, HOE) or six (WJ) randomly located zero tension lysimeters per plot. They consisted of a 5 cm high stainless steel box covered with a nylon mesh (1 mm² mesh size), installed just below the forest floor. Soil solution from the mineral soil was sampled with ceramic cup suction lysimeters (Eijkelkamp) at three locations per plot. Each location was equipped with two to four lysimeters at

to a series of 200 L polyethylene storage containers mounted in a cascade system. The volume collected by each individual tree was determined at every sampling event. Subsamples were taken from all full containers, with subsample volumes weighted to tree diameters, and bulked to one sample. Stemflow volumes, obtained from the individual trees, were upscaled to plot level using information of basal area.

Table 1
Characteristics of the five Level II plots. Mean annual temperature (MAT) and precipitation (MAP) are long-term averages for the nearest meteorological station (1981–2010, Royal Meteorological Institute of Belgium, www.meteo.be). Basal area was calculated from a full survey (DBH ≥ 5 cm) in 2009–2010. Humus types were defined in 2007 (Zanella et al., 2011). Total organic N stock is given for the forest floor (FFN) and the upper 1 m of the mineral soil (SON) (Peck et al., submitted for publication).

<table>
<thead>
<tr>
<th>Plot</th>
<th>Coordinates</th>
<th>Elevation (m)</th>
<th>MAT (°C)</th>
<th>MAP (mm)</th>
<th>Tree species</th>
<th>Planting year</th>
<th>Former use</th>
<th>Basal area (m² ha⁻¹)</th>
<th>Humus type</th>
<th>FFN (ton N ha⁻¹)</th>
<th>SON (ton N ha⁻¹)</th>
</tr>
</thead>
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<tr>
<td>RAV</td>
<td>51°24'07&quot;</td>
<td>05°03'15&quot;</td>
<td>35</td>
<td>10.4</td>
<td>Pinus nigra ssp. laricio var. Corsicana Loud.</td>
<td>1930</td>
<td>Heath</td>
<td>44.9</td>
<td>Mor</td>
<td>1.58</td>
<td>5.2</td>
</tr>
<tr>
<td>BRA</td>
<td>51°18'28&quot;</td>
<td>04°31'11&quot;</td>
<td>14</td>
<td>10.8</td>
<td>Pinus sylvestris L.</td>
<td>1929</td>
<td>29.2</td>
<td>Mor</td>
<td>1.26</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>WIJ</td>
<td>51°04'11&quot;</td>
<td>03°02'14&quot;</td>
<td>31</td>
<td>11.0</td>
<td>Fagus sylvatica L.</td>
<td>1935</td>
<td>Arable</td>
<td>36.5</td>
<td>Mor</td>
<td>2.67</td>
<td>10.4</td>
</tr>
<tr>
<td>GON</td>
<td>50°58'31&quot;</td>
<td>03°48'15&quot;</td>
<td>26</td>
<td>10.6</td>
<td>Quercus robur L., Fagus sylvatica L.</td>
<td>1918</td>
<td>Old growth</td>
<td>31.9</td>
<td>Moder</td>
<td>1.58</td>
<td>10.9</td>
</tr>
<tr>
<td>HOE</td>
<td>50°44'45&quot;</td>
<td>04°24'47&quot;</td>
<td>129</td>
<td>10.7</td>
<td>Fagus sylvatica L.</td>
<td>1909</td>
<td>Old growth</td>
<td>28.9</td>
<td>Moder</td>
<td>0.84</td>
<td>7.7</td>
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</table>

Table 2
Soil characteristics of the five Level II plots: soil type according to IUSS Working Group WRB (2007), sampling depths, morphogenetic horizons, C:N ratio, pH-CaCl₂ (molarity 0.01 M), cation exchange capacity (CEC) and base saturation (BS) for the forest floor layers (OF, OH or OFH) and five fixed depth layers of the mineral soil. Soil texture data are given for the mineral soil layers (clay, silt and sand fractions in mass %). Soil samples were analysed using methods of the ICP Forests manual (ICP Forests, 2010).

<table>
<thead>
<tr>
<th>Plot</th>
<th>Soil type</th>
<th>Depth (cm)</th>
<th>Morphogenetic Horizon</th>
<th>C:N</th>
<th>pH-CaCl₂ (molarity 0.01 M)</th>
<th>CEC cmol kg⁻¹</th>
<th>BS %</th>
<th>Clay 0–2 µm</th>
<th>Silt 2–63 µm</th>
<th>Sand 63–2000 µm</th>
</tr>
</thead>
<tbody>
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<td>RAV</td>
<td>Endogleyic folic brunic</td>
<td>–6.0 to 0.8</td>
<td>OF</td>
<td>33</td>
<td>2.5</td>
<td>23</td>
<td>36</td>
<td></td>
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<td></td>
<td>Albic arenosol (dystric)</td>
<td>–0.8–0</td>
<td>Ap/E</td>
<td>27</td>
<td>2.9</td>
<td>46</td>
<td>11.2</td>
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<td></td>
<td></td>
<td>0–5</td>
<td>Ap/E</td>
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<td>3.0</td>
<td>7.6</td>
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<td>10–20</td>
<td>Ap/E</td>
<td>23</td>
<td>3.1</td>
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<td>0.7</td>
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<td>0.4</td>
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<td></td>
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<td>B, Bg1</td>
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<td>40–80</td>
<td>Btx1</td>
<td>5.4</td>
<td>3.8</td>
<td>5.2</td>
<td>16</td>
<td>19.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Concentrations in a given year were interpolated using a logarithmic curve for all measured DOC concentrations in function of sampling period number in that year. In case not enough data were available or DOC concentrations were still high in January following the peak of DOC concentrations at the end of the previous year, DOC concentrations were calculated as the average concentration of the measurements preceding and following the missing value. For soil solution DON and DOC concentrations measured in a period without rainfall, no drainage flux could be determined. In that case the drainage flux was interpolated as the average of the previous and the following drainage flux obtained from measured concentrations, if possible weighted by concentration and sample volume. We assumed that DON fluxes through the C horizon represented organic N losses below the rooting zone and thus lost from the ecosystem.

2.4. Data handling

Concentrations of DOC were taken from a previous study for 2005–2012 (Verstraeten et al., 2014), and were supplemented with new data for 2013. Concentrations of DON were calculated as TKN minus total Na+ concentrations in the soil solution. Using a TOC-analyser (Shimadzu TOC 5050 A, LOQ = 0.1 mg L\(^{-1}\)) and sodium (Na\(^{+}\)) and ammonium (NH\(_4\)\(^{+}\)) were determined using ion chromatography (Dionex ICS–3000, LOQ = 0.1 mg L\(^{-1}\)). Concentrations of DOC were determined using a TOC-analyser (Shimadzu TOC 5050 A, LOQ = 0.1 mg L\(^{-1}\)). pH was measured on unfiltered subsamples (Multi 340i-glass electrode, WTW).

2.5. Statistical analysis

Data exploration and statistical analysis were performed in R (R Core Team, 2014). The nonparametric Seasonal Mann-Kendall Test (Hirsch et al., 1982) included in the ‘rkt’ package (Marchetto, 2015) was applied to detect monotonic trends in DON concentrations, DON fluxes, DOC:DON, pH (2005–2013) and temperature (1996–2013). The rate of annual change (increase or decrease) was expressed as a percentage in function of theil-Sen’s slope (% per year). Since DON concentrations were not normally distributed (evaluated using the Shapiro-Wilk test included in the ‘stats’ package), cross-site statistics (for inter-comparison of sites) were performed by means of the non-parametric Kruskal test (Multiple comparison test after Kruskal–Wallis) included in the ‘pgirmess’ package (Giraudoux, 2015). Correlations between fortnightly measured concentrations of DOC or DON and mean air or soil temperature during the period since the previous sampling were evaluated using the Spearman’s rank correlation test included in the ‘stats’ package.

3. Results

3.1. Trends and patterns of DON

In BD, mean annual concentrations (0.40–0.48 mg L\(^{-1}\)) and fluxes (3.0–3.9 kg ha\(^{-1}\) y\(^{-1}\)) of DON were comparable among the five studied Level II plots (Table 3). In TF, mean annual concentrations (0.95–1.41 mg L\(^{-1}\)) and fluxes (5.6–8.3 kg ha\(^{-1}\) y\(^{-1}\)) of DON were respectively 2.8 and 1.9 times higher than in BD. Fluxes of DON in TF were somewhat higher in the coniferous plots. In the O horizon we observed the highest mean annual DON concentrations (1.84–2.36 mg L\(^{-1}\)) and fluxes (10.1–12.3 kg ha\(^{-1}\) y\(^{-1}\)). In the mineral soil, DON levels decreased gradually with soil depth from the A till the C horizon. Annual leaching losses of DON (i.e. leaching of DON from the C horizon) were 1.2–3.7 kg ha\(^{-1}\) y\(^{-1}\), representing <0.1% of the total soil organic N stock to 1 m depth (Table 1).

Concentrations and fluxes of DON in BD were slightly higher during the growing season (from May to August) compared to the rest of the year (Figs. 1 and 2). In TF we observed a more explicit seasonal pattern, with a marked peak of DON concentrations in May, especially in the deciduous plots, and elevated DON fluxes from May till August in all plots. In the soil solution the highest DON fluxes were observed at the end of the year, i.e. when rainfall was abundant.

The amount of precipitation and the drainage fluxes exhibited a certain inter-annual variation (Fig. A.1), but did not significantly change between 2005 and 2013 (Table 3). Concentrations and fluxes of DON in BD were stable (Table 3, Figs. A.2 and A.3). In TF, DON concentrations and fluxes increased significantly in all plots by 3–9% per year, except fluxes in RAV. In the O horizon, a general increase of DON concentrations (7–19% per year) and DON fluxes (4–14% per year) was observed. Also in the A, B and C horizons, the highest mean annual DON concentrations (1.84–2.36 mg L\(^{-1}\)) and fluxes (10.1–12.3 kg ha\(^{-1}\) y\(^{-1}\)) of DON were calculated as the product of mass balance method, which is originally based on the assumption of conservation of mass between the input of atmospheric Cl\(^{-}\) and the Cl\(^{-}\) flux in the subsoil (Eriksson and Khunakasem, 1969). Because the conservative behaviour of Cl\(^{-}\) is subject to discussion (Svensson et al., 2012), we used Na\(^{+}\) as a tracer instead of Cl\(^{-}\) (De Schrijver et al., 2004, 2008). The mass balance method requires the assumption of conservative behaviour of Na\(^{+}\) in the soils. For the plots studied, we assumed that the contribution of weathering to Na\(^{+}\) concentrations in the soil solution is negligible over the study period, given the minor amounts of Na-Feldspar and mica in the finer soil textures and the absence of these minerals in the sandy soils (Van Ranst et al., 2002). We also assumed that the amount of exchangeable Na\(^{+}\) and the adsorption of Na\(^{+}\) to clay mineral surfaces are negligible, given the very low soil pH (Table 2) and the absence of clay minerals with permanent surface charge (Van Ranst et al., 2002). Soil solution DON and DOC fluxes (kg ha\(^{-1}\)) for the fortnightly sampling periods were calculated as the product of water flux (L m\(^{-2}\) h\(^{-1}\)) and concentration (mg L\(^{-1}\)). Monthly DON and DOC fluxes were calculated as the sum of the two fortnightly fluxes and annual fluxes as the sum of the twelve monthly fluxes. Missing data (for several evident reasons e.g., no sampling performed due to snow, or insufficient sample volume available for analysis) would result in an underestimation of DON and DOC fluxes. Missing concentrations were interpolated to correct for this as follows. DOC concentrations in a given year were interpolated using a logarithmic interpolation method (throughfall + stemflow further denoted as TF). Drainage fluxes were calculated for each depth using the mass balance method, which is originally based on the assumption of conservation of mass between the input of atmospheric Cl\(^{-}\) and the Cl\(^{-}\) flux in the subsoil (Eriksson and Khunakasem, 1969). Because the conservative behaviour of Cl\(^{-}\) is subject to discussion (Svensson et al., 2012), we used Na\(^{+}\) as a tracer instead of Cl\(^{-}\) (De Schrijver et al., 2004, 2008). The mass balance method requires the assumption of conservative behaviour of Na\(^{+}\) in the soils. For the plots studied, we assumed that the contribution of weathering to Na\(^{+}\) concentrations in the soil solution is negligible over the study period, given the minor amounts of Na-Feldspar and mica in the finer soil textures and the absence of these minerals in the sandy soils (Van Ranst et al., 2002). We also assumed that the amount of exchangeable Na\(^{+}\) and the adsorption of Na\(^{+}\) to clay mineral surfaces are negligible, given the very low soil pH (Table 2) and the absence of clay minerals with permanent surface charge (Van Ranst et al., 2002). Soil solution DON and DOC fluxes (kg ha\(^{-1}\)) for the fortnightly sampling periods were calculated as the product of water flux (L m\(^{-2}\) h\(^{-1}\)) and concentration (mg L\(^{-1}\)). Monthly DON and DOC fluxes were calculated as the sum of the two fortnightly fluxes and annual fluxes as the sum of the twelve monthly fluxes. Missing data (for several evident reasons e.g., no sampling performed due to snow, or insufficient sample volume available for analysis) would result in an underestimation of DON and DOC fluxes. Missing concentrations were interpolated to correct for this as follows. DOC concentrations in a given year were interpolated using a logarithmic curve for all measured DOC concentrations in function of sampling period number in that year. In case not enough data were available or DOC concentrations were still high in January following the peak of DOC concentrations at the end of the previous year, DOC concentrations were calculated as the average concentration of the measurements preceding and following the missing value. For soil solution DON and DOC concentrations measured in a period without rainfall, no drainage flux could be determined. In that case the drainage flux was interpolated as the average of the previous and the following drainage flux obtained from measured concentrations, if possible weighted by concentration and sample volume. We assumed that DON fluxes through the C horizon represented organic N losses below the rooting zone and thus lost from the ecosystem.
horizons DON concentrations and fluxes generally tended to increase, but the magnitude of the increase and the number of significant differences diminished gradually with soil depth. Only at the HOE site a significant increase in DON losses below the rooting zone was observed.

At the BRA site, the concentrations of DON and DOC in TF showed a strong positive correlation \( (p < 0.001) \) with air temperature. Soil solution DON concentrations in the A horizon also showed a strong positive correlation \( (p < 0.001) \) with soil temperature, while soil solution DON concentrations in the A horizon were not correlated with soil temperature. There was no significant change in temperature during the observation period (Fig. A.4).

### 3.2. Trends and patterns of DOC:DON

In BD, DOC:DON was comparable among plots (median 5.9–6.8) (Table 3). In TF, DOC:DON was higher in coniferous plots (median 15.3–16.3) than in deciduous plots (median 9.4–10.3). In the soil solution, DOC:DON increased with depth from the O horizon (median 20.4–25.6) to the A horizon (median 28.3–34.8) in all plots except GON (median 18.3–16.3); in the mineral soil, DOC:DON decreased with depth (median 15.3–24.0) in the C horizon) in all plots. Overall, DOC:DON in soil solution was higher in the plots with a sandy soil texture (RAV, BRA, WIJ) than in the plots with a more silty loam texture (Table 2). In TF and in the O horizon, DOC:DON was somewhat higher near the end of the growing season, while DOC:DON showed no clear seasonal pattern in BD and in the mineral soil (Fig. 3).

The concentrations of DOC and DON were weakly correlated in BD, while a strong correlation was observed in TF at three plots (Table 3). In the soil solution, concentrations of DOC and DON showed an overall strong correlation, except in the A horizon.

In BD, DOC:DON remained nearly stable between 2005 and 2013, while DOC:DON decreased overall in TF and in the soil solution (Table 3). The DOC:DON ratio in soil solution peaked in 2007, followed by more or less stable values thereafter. The concentrations of DOC and DON were weakly correlated in BD, while a strong correlation was observed in TF at three plots (Table 3). In the soil solution, concentrations of DOC and DON showed an overall strong correlation, except in the A horizon.

In BD, DOC:DON remained nearly stable between 2005 and 2013, while DOC:DON decreased overall in TF and in the soil solution (Table 3). The DOC:DON ratio in soil solution peaked in 2007, followed by more or less stable values thereafter.

### Soil solution pH

Soil solution pH showed an overall increasing trend between 2005 and 2013, followed by more or less stable values thereafter.

### 4. Discussion

Despite the wide scientific interest for DON concentrations and fluxes in temperate forests during the past decades (Kabitz et al., 2016), the results presented here indicate that the impact of climate change on DON fluxes and the associated biological processes is complex and depends on multiple factors, such as soil type, vegetation, and management practices.
monitoring of DON concentrations was implemented only recently in forest monitoring networks. The number of published long-term data series on DON concentrations and fluxes is therefore very limited and published time series were often shorter than the minimum of nine years that is recommended to be able to detect clear trends (Oulehle et al., 2011; Vanguelova et al., 2010; Waldner et al., 2014). Our unique 9-year time series of DON concentrations and fluxes in five temperate forests in a high deposition area brings new insights into this matter.

Overall, the concentrations and fluxes of DON in the deposition and soil solution of the five plots were in the middle or higher range of the levels that have been observed at other temperate forests in northwestern and central Europe and eastern US (Michalzik et al., 2001; Oulehle et al., 2011; Sleutel et al., 2009; Vanguelova et al., 2010; Wu et al., 2010). The DOC:DON ratios that were observed in BD were in the lower range of those reported in similar published studies (Campbell et al., 2000; Michalzik and Matzner, 1999; Solinger et al., 2001), probably due to the relatively high atmospheric DON depositions. The higher DOC:DON in TF in coniferous plots compared to deciduous plots could be attributed to the higher C:N ratio of needles compared to leaves (Cools et al., 2014).

The concentrations and fluxes of DON were stable in BD, but increased in TF during the monitoring period, in contrast with the increase of DOC concentrations in BD and the stable DOC concentrations and fluxes in TF that was observed at the five plots between 2002 and 2012 (Verstraeten et al., 2014). On the other hand, we observed a parallel increase in DON and DOC levels in soil solution. Hence, our hypothesis that trends of DON follow the trends of DOC, was confirmed for soil solution but not for deposition.

The observed increase of DON concentrations and fluxes in the soil solution is in agreement with the predominantly increasing trends of DOC levels observed in forest soils and connected aquatic ecosystems in the central parts of Europe and eastern US (e.g. de Wit et al., 2007; Monteith et al., 2007; Oulehle et al., 2011. Vanguelova et al., 2010). DOC and DON form a crucial link between terrestrial and aquatic carbon and N cycles and contribute significantly to atmospheric deposition.

![Fig. 1. Mean monthly DON concentration (mg L⁻¹) in deposition and soil solution (2005–2013) with 95% confidence interval based on the propagation of errors (dotted bars).](image-url)
aquatic fluxes of carbon dioxide (CO₂) to the atmosphere (Battin et al., 2009; Lapierre et al., 2013; Regnier et al., 2013). But annual leaching losses of DON below the deepest horizon considered (1.2–3.7 kg ha⁻¹ y⁻¹) represented <0.1% of the total soil organic N stock to 1 m depth and increased only at one of the five plots. Moreover, DON leaching losses were much lower than DOC losses which amounted to 19–61 kg ha⁻¹ y⁻¹ at the five plots (Verstraeten et al., 2014), meaning that the impact of DON leaching on the water quality of connected surface waters is probably limited compared to DOC leaching.

A possible explanation for the increase of DON and DOC levels is the significant decline of atmospheric SO₄²⁻ depositions at the five plots between 1994 and 2010 (Verstraeten et al., 2012). At a number of intensive monitoring plots in European temperate forests, including the five Level II plots studied, the decline of atmospheric SO₄²⁻ depositions was followed by an initial chemical recovery of the soil solution, indicated by an increase of pH (Fig. A.6) and a decrease of SO₄²⁻ and Al³⁺ concentrations (Oulehle et al., 2011; Vanguelova et al., 2010; Verstraeten et al., 2012). As postulated by Monteith et al. (2007) decreasing soil solution SO₄²⁻ concentrations result in lower soil solution ionic strength, leading to an increased solubility of DOM. In addition, ionic Al in soil is known to coagulate DOM, thereby causing precipitation of DOM. Increasing pH therefore also indirectly increases DOM solubility by lowering soil solution concentration of Al³⁺. At the low pH-CaCl₂ of about 3 of the mineral horizons (Table 2), i.e. in the low end of the Al buffer range, a relatively small increase in pH could strongly reduce Al solubility and therefore promote DOM solubility. Hence, the increase of DON in soil solution could be attributed to multiple interlinked abiotic processes, viz. the increase in pH, lowered soil solution Al³⁺ levels and lower SO₄²⁻ concentrations and ionic strength. Trends in DOC:DON were mostly negative, disproving our hypothesis that recovery from acidification would disproportionally favor the dissolution of DOC relative to DON.

The level of N loading was found to be positively correlated with DON concentration and negatively with DOC:DON, suggesting that

\[ \text{Fig. 2. Mean monthly DON flux (kg ha}^{-1} \text{ month}^{-1}) \text{ in deposition and soil solution (2005–2013) with 95% confidence interval based on the propagation of errors (dotted bars).} \]
Inorganic N can be transformed into DON, as previously postulated (Brookshire et al., 2007; Campbell et al., 2000; Currie et al., 1996; McDowell et al., 2004; Pregitzer et al., 2004). Inorganic N deposition and NO₃⁻ leaching decreased at the five plots (Verstraeten et al., 2012). Since we observed increasing trends of DON and mostly decreasing DOC:DON in TF and soil solution, we found no cues that DON levels and DOC:DON ratios were influenced by inorganic N deposition at the five plots.

DON was furthermore enriched in DOM with depth and followed the decrease of the soil C:N ratio with depth (Table 2). Considering the very low unfavorable pH of the soil mineral horizons (pH-CaCl₂ was close to 3), it seems unlikely that microbial consumption of C explains decreasing DOC:DON ratios with depth. Instead, progressively lower DOC:DON ratio is likely explained by selective sorption of DOC upon leaching through mineral soil horizons, because N-rich DOM compounds often have a lower reactivity with iron (Fe) and Al hydroxides compared to N-poor DOM compounds (Qualls et al., 1991; Scott and Rothstein, 2014; Vandenbruwane et al., 2007). In line, at the GON site, the overall low DOC:DON ratios match the more loamy soil texture with 4–10 times higher cation exchange capacity, and thus higher sorption capacity of the soil compared to the other plots with a more sandy texture (Table 2).

DOM composition was also influenced by temporal variation in hydrological conditions. The DOC:DON ratio in soil solution peaked in 2007 (Fig. A.5), a year characterized by exceptional spring drought followed by abundant rainfall during summer. Clearly in all five sites this sudden rewetting after 37 days of drying temporarily promoted DOC losses but lowered DON losses. Considering predicted global changes in precipitation and evapotranspiration, soil drying and rewetting will likely increase in the next decades (Borken and Matzner, 2009), with possible high temporal DOC leaching.

Although DOC and DON concentrations in water samples are negatively correlated with precipitation through dilution (Michalzik and Matzner, 1999; Sleutel et al., 2009), the seasonal peak in May of DON concentrations in TF in the deciduous plots could not be explained by
differences in the monthly precipitation (Fig. A.1). This peak coincided with the dispersal of N-rich pollen, bud burst and the fall of bud scales from oak and beech trees, which could explain the darker, brownish color of water samples from throughfall and stemflow during this month. Fluxes of DON in TF depended strongly on precipitation, which was highest in August and December and lowest in January and April (Fig. A.1), in accordance with the 30-year average of monthly precipitation in Belgium (www.meteo.be). Our results confirmed that the canopy functions as an important source of DON and DOC in temperate forests (Michalzik et al., 2001), and we observed that particularly spring phenology had a considerable impact on throughfall DON concentrations and fluxes in deciduous forests, while the impact of autumn phenology (leaf senescence and fall), typically around October–November, was more limited. Given that DON fluxes showed no trend in BD, suggesting stable atmospheric DON inputs, the increase of DON levels in TF could likely be explained by intensified DON leaching from the canopy. The positive correlation between DOC and DON concentrations in TF and air temperatures at the BRA site confirmed the role of air temperature as a driver of DOM in throughfall water (Sleutel et al., 2009; Solinger et al., 2001; Wu et al., 2010). A possible explanation is that higher temperatures may stimulate microbial activity and insect herbivore activity in the canopy, which are known to enhance DOM leaching with throughfall (Michalzik and Stadler, 2005; Pitman et al., 2010). Therefore, a future with climate warming could result in higher DOM leaching from the canopy. The DOM in throughfall consists for a large part of easily decomposable compounds, which could act as co-substrates or promoters for decomposition and mineralization processes of organic matter in the forest floor (Guggenberger and Zech, 1994). Therefore, increased leaching of DON from the canopy may help to explain the increase of DON levels in the upper soil layers (Michalzik et al., 2001; Michalzik and Stadler, 2005). Higher soil temperatures may in turn enhance the leaching of DOM from organic soil layers (Andersson et al., 2000; Michalzik et al., 2001). In our study, this was only confirmed for DOC but not for DON, given the non-significant correlation between soil solution DON concentrations and soil temperature in the A horizon at the BRA site.

5. Conclusions

Following a sharp decline in atmospheric SO$_2^-$ and inorganic N depositions during the past two decades, forests in Flanders, the Northern part of Belgium, are under recovery from acidification. The concentrations and fluxes of DON in soil solution at five ICP Forests Level II plots showed increasing trends over a 9-year period starting in 2005. This mobilization of DON connects to the predominantly increasing trends of DOC levels observed in forest soils in the central parts of Europe and eastern US. The increase of DON in soil solution can be attributed to multiple interlinked abiotic processes, viz. increase in pH, decrease in soil solution Al$^{3+}$ and SO$_4^{2-}$ concentrations and ionic strength. But further in-depth statistical analysis able to cope with the multicollinearity among atmospheric deposition, soil solution, pH and DON level data will be needed. Such exercise requires long-term high-resolution datasets like the one presented here. Given the decrease in inorganic N deposition over the monitoring period, the generally decreasing trend in DOC:DON ratio in TF and soil solution does not appear to result from an altered N-balance. Also, no major shifts in air temperature were observed. We therefore argue that recovery from acidification may be the most plausible explanation for observed shifts in DON and DOC:DON trends over this 9-year period. Whether or not the observed trends and patterns of DON also apply to a wider spatial scale will need to be further investigated, including its dependency on factors like latitude, level of N deposition, and hydrology.

Acknowledgments

This work was based on monitoring co-financed by the European Commission under regulations (EC) No 3528/86, Forest Focus (EC) No 2152/2003 for data from 2005 and 2006, FutMon (EC) LIFE07 ENV/D/218 for data from 2009 till June 2011, and the Flemish Government. We would like to thank everyone who assisted with the collection and analysis of samples. Our special thanks go to Thierry Onkelinx for his advice on statistical analysis of the data.
Fig. A.2. Fortnightly DON concentration (mg L$^{-1}$) in deposition and soil solution, with trend lines (blue: linear regression line, red: LOESS curve).

Fig. A.3. Fortnightly DON flux (kg ha$^{-1}$ fortnight$^{-1}$) in deposition and soil solution, with trend lines (blue: linear regression line, red: LOESS curve).
Fig. A.4. Daily mean air temperature (black dots) and soil temperature in the A horizon (blue: 2 cm depth, red: 9 cm depth) at the BRA site (1996–2013). Dashed line: mean air temperature for the entire period (10.67 °C), solid green line: linear regression line for air temperature (with 95% confidence interval in light green), solid black line: gam model (air temperature ~ s(date, bs = "cs")) for air temperature (with 95% confidence interval in grey) and significance of Seasonal Mann-Kendall trends (ns: not significant).

Fig. A.5. Fortnightly DOC:DON in deposition and soil solution, with trend lines (blue: linear regression line, red: LOESS curve).
Fig. A.6. Soil solution pH (2005–2013) and significance of Seasonal Mann-Kendall trends (ns: not significant, (+): p < 0.1, (+): p < 0.05, ++: p < 0.01, +++: p < 0.001), with trend lines (blue: linear regression line, red: LOESS curve).

Fig. A.7. Fortnightly DOC concentration (mg L$^{-1}$) in deposition and soil solution, with trend lines (blue: linear regression line, red: LOESS curve).