Temperature, wetting cycles and soil texture effects on carbon and nitrogen dynamics in stabilized earthworm casts

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Accepted 9 September 1999

Abstract

Two soils, one a silty loam soil (Kilkea) and the other a clayey soil (Leitrim), and stabilized earthworm cast derived from both soils, were subjected to two wet-drying regimes (4 d and 16 d wetting intervals) at two temperatures (10°C and 20°C). Each treatment was sampled regularly for CO2 emissions and dissolved organic carbon, ammonium and nitrate were measured in leachate. Production of CO2 from the clayey soil was 20–40% less than from the silty loam at both temperatures and under both cycles, while production was 40% less at the lower temperature. Amounts of dissolved organic carbon (DOC) in leachate from the silty loam soil were greater than from the clayey soil, while, in general, greater amounts of DOC leached from uningested soil than from earthworm cast. Treatments with the silty loam soil lost approximately 30–40 times more total N than corresponding clayey soils under the 4 d cycle, and 12–16 times more under the 16 d cycle, thus illustrating the extent of nutrient protection in clay soils, but also the reduction of this effect with super-saturation and prolonged drying (16 d cycle). Nutrient losses suggest greater protection of nutrients in cast at first, followed by a reduction in protection (weakening of aggregate structure) in later cycles. These results have important implications for nutrient losses in the field, since significant and complex interactions were evident between the experimental factors. To improve our understanding of soil organic matter (SOM) dynamics, interactions between climatic, soil and cast-mediated changes (i.e. increased organic–inorganic interaction and burial) need to be incorporated into future models of soil decomposition.

Keywords: Earthworms; Wet-drying cycles; Microaggregation; Organic matter; Stabilization; Carbon; nitrogen; Leachate; Dissolved organic carbon; Respiration

1. Introduction

Many factors, which operate at vastly different spatial and temporal scales (Wardle and Lavelle, 1997), affect the sizes of soil organic carbon pools and global CO2 flux (Bolin et al., 1979; Swift et al., 1979; Stevenson, 1986). The role of soil texture (silt/clay content) in the formation of organic–inorganic complexes and soil aggregates, and the protection of organic compounds from biological degradation has long been appreciated (Mortland, 1970). By slowing and modifying humic transformations (Anderson et al., 1981) soils with higher silt/clay content retain higher proportions of residual organic C and N (Jenkinson, 1971; Sorensen, 1972; Ladd et al., 1975).

Increasing silt/clay content also increases water holding capacity, so that soil texture interacts with climate in controlling these ecosystem processes (Burke et al., 1989). This interactive complexity is further increased by earthworm cast production and its potential for variability with age and state.

Microaggregates containing a core of organic matter (Shipitalo and Protz, 1989) are formed during transit through the earthworm’s gut and once these organic–inorganic matrices are egested as casts, bonds between organic and mineral components are strengthened when these materials are brought into close association, due to water loss. Thus earthworm cast struc-
ture can enhance aggregation (Blanchart et al., 1990), structural stability (De Vleeschauwer and Lal, 1981) and tensile strength (McKenzie and Dexter, 1987; Zhang and Schrader, 1993) of soils. However, this stability is greatly influenced by water content. Not surprisingly, studies on fresh casts (Shipitalo and Protz, 1988; Marinissen and Dexter, 1990; Hindell et al., 1994a,b) have pointed out that their physical properties, particularly relating to structural stability, can vary with age and wetting/drying cycles. Thus the compact structure of casts may result in temporary immobilisation, but also under certain moisture/temperature conditions, the pulsed release of nutrients (Lavelle and Martin, 1992; Wardle and Lavelle, 1997). Marinissen and Dexter (1990) found that one drying/rewetting cycle increased the stabilisation of casts. Hindell et al. (1994b) concluded that the wetter the soil and the longer it stays wet, the longer casts remain in a dispersible state, and that this could increase nutrient losses. The microstructural state of casts can also be influenced by their position (buried or surface) in the soil, since buried casts can remain in a more moist condition than surface casts. In relation to this, Oades (1993) noted that earthworms influence the rate, extent and spatial development of the soil drying phase. Thus, over the medium- to long-term, cast structure may enhance (O’Brien and Stout, 1978) or block (Martin, 1991) the overall mineralization of soil organic matter. With large volumes of soil being ingested by earthworms (Lavelle, 1978; Lee, 1983) and given that the casts differ physically and chemically (and microbiologically) from the soil from which they are derived they represent an important source of soil heterogeneity (Anderson, 1988).

Previous studies have focused on short-term physical and chemical changes in fresh casts. Few have concentrated on aged, stabilised casts, though the potential for cast persistence in soil has been well documented (Lavelle, 1978; Lee, 1983). We examined the interactions between wetting/drying cycles, temperature, silt/clay content on aged and already stabilised earthworm casts (i.e. medium to long-term decomposition) and the subsequent C and N dynamics of these soil aggregates. In particular it examined the extent to which respired carbon (CO₂-C) and dissolved organic carbon (DOC) were affected by these treatments.

2. Materials and methods

2.1. Soils

The soils we used were collected from the top 7–15 cm of the mineral horizons at two sites in Ireland. The Kilkea soil (Typic Hapludalf) was taken from a Norway spruce stand in Kilkea, Co. Kildare. It had a pH of 4.7, water holding capacity of 36.0%, a C content of 3.825%, total N content of 0.315% and an organic matter content of 8.62%. The particle sizes were 20.81% clay, 38.42% silt and 40.77% sand. The Leitrim soil was a heavy gley soil (Gley, Alfic Hapludalf) from a Sitka spruce forest site at Lisgavin near, Co. Leitrim. The soil had a pH of 4.8, water holding capacity of 29.8%, C content of 2.41%, total N content of 0.182% and an organic matter content of 5.83%. The soil contained 48.17% clay, 42.75% silt and 9.08% sand. Prior to their use in these experiments the soils were air dried, homogenised (macroaggregates broken up, large roots, macro-organic particles and stones removed) and sieved (2 mm).

2.2. Earthworm cast and control soil production

Earthworms (Lumbricus terrestris Linneaus) exhibited normal feeding and burrowing behaviour while being maintained at a mean temperature of 15 ± 3°C and 80–100%RH in containers with oak leaf litter (Quercus petraea Mattuschka, Liebl.) and soil. The resulting casts were collected and stored at 4°C until the experiments began. Earthworm casts produced using the Kilkea soil had an organic matter content 1.17% greater than the initial soil. To ensure that non-cast treatments had a similar organic matter content, shredded oak litter was incorporated by gentle hand mixing of soil, water and oak (shredded to <2 mm) to form macroaggregates. The resulting mixture for both cast and control soil contained 9.79% organic matter. A similar procedure was adopted for Leitrim soils (6.99% organic matter, 1.48% derived from incorporated litter). Oak litter, cast and parent soil organic matter contents were estimated as ‘loss-on-ignition’. The percentage of clay, silt and sand in earthworm cast was found to be similar to that of uningested soil and aggregate structure of both casts and uningested soil (controls) was similar (dia 0.4–1.3 cm approximately).

2.3. Incubation units and procedure

Carbon, nitrogen and water fluxes were examined using laboratory based incubation units. These transparent polystyrene Nalgene filtration units (Cat. No. FD240-10), consisted of an upper chamber, used to hold the soil and sand layers, and a lower chamber where leachate was collected. Eighty grams of cast or control soil (derived from Kilkea and Leitrim soils) were added to these units above a 20 g layer of acid washed sand (i.e. bottom layer).

The unit was sealed to ensure that it was completely air tight during the respiration measurements. A head space of approximately 75 ml was present above the soil in the upper chamber. Gas may have diffused
through the soil and sand layers into the lower chamber but such losses were expected to be minimal.

A 40 μm filter separated the unit chambers thus preventing sand from passing through. It was envisaged that the sand in turn would prevent larger particulate organic carbon (POC) from leaching, but allow dissolved organic carbon (DOC) to percolate into the lower chamber of the experiment unit.

The cast and control soils were stored for 6 weeks at 14°C with a water content of 60–80% water holding capacity. This was to ensure that the initial short term pulse of mineralization associated with fresh earthworm casts had ended, and that steady state, medium- to long-term mineralization rates were in effect. The cast and control were then dried at 20°C for 2 d and stored at 4°C. This enabled a similar conditioning incubation to be set for all treatments.

To ensure that drying and cold storage did not adversely affect microbial development during the experiment, a comparison of CO₂ production (at 20°C and 60–80% water holding capacity) for nonstored and dried/cold stored/reinoculated treatments was carried out. No significant differences were evident between these treatments after 10–12 d. Thus, all treatments were maintained at 20°C and 60–80% water holding capacity for a period of 14–16 d before the experiment began.

In total, 16 treatments, with 4 replicates, were used in this experiment as outlined in Table 1. Cast and control soil treatments derived from both the Kilkea and Leitrim soils were placed in incubation units, which were then subject to two wet/drying regimes (4 d and 16 d wetting intervals) and two temperature regimes (10°C and 20°C).

For the 4 d cycle, using a Pasteur pipette to simulate rainfall (droplet only), 30 ml of distilled water were added at the beginning of each cycle, while 120 ml were added at the start of each 16 d cycle. Thus an equal amount of water was added under either cycle over a 16 d period. The experiment lasted for 80 d for all treatments, that is, five 16 d cycles and twenty 4 d cycles. Each treatment was sampled regularly for CO₂ emissions while DOC, ammonium and nitrate were measured in leachate.

2.4. Analyses

Moisture content (%) was estimated by drying at 105°C (Allen, 1989; Anderson and Ingram, 1993). ‘Loss-on-ignition’ was used to estimate percentage organic matter (Allen, 1989). Samples of 2–4 g were dried at 105°C, ground for 10 min at 900 oscillations min⁻¹ using a RETSCH grinder (type MM2), before total C, H and N of cast and soils were measured by flash combustion followed by GC using a CARL-ERBA 1106 automatic analyser.

CO₂ production was measured by syringe removal of a 5 ml air sample from the experiment units’ head space (75 ml) after a 15–20 min incubation. The samples were then injected into a modified Ciras-SC (PP Systems) infra red gas analyser. Samples were taken 3 times during the first 24 h (2, 4 and 8 h after wetting), and on the second (24 h) and third (48 h) days, after wetting. For the 16 d wetting interval, CO₂ was further sampled at 2–4 d, 4–8 d after the wetting phase and on d 14 of the cycle.

Leachate was collected approximately 2 h after the end of the water application i.e. when no more water droplets were coming from upper chamber of the units. Immediately after the sample was taken, DOC (in leachate filtered through a 0.45 μm Millipore (HAWP 01300)) was estimated by measurement of absorbence at 400 nm and comparison with a standard curve obtained using a TOC analyser. Samples were then frozen for a maximum of 2 weeks before concentrations of nitrate-N, chloride and sulphate were measured using ion chromatography (Dionex Qic AS4A separator column) and concentrations of ammonia-N were measured using a Tecator Flow Injection Analyser FIAstar 5010 using the method described in application notes ASN 50-01/84. Samples were filtered through a 0.2 μm membrane filter to remove particulate matter and organic material. Three replicates were analysed for ammonia-N and nitrate-N, and four replicates were sampled for DOC. The results were analysed using a four fixed factor (temperature, wetting cycle, cast and soil type) model of analysis of variance. All analysis was carried out using SAS (Statistical Analysis Software, 1992).

All the results are expressed as amounts g⁻¹ soil car-

### Table 1

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Temperature (°C)</th>
<th>Wetting cycle (d)</th>
<th>Cast/no cast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kilkea 10</td>
<td>4</td>
<td>cast</td>
<td></td>
</tr>
<tr>
<td>Kilkea 10</td>
<td>16</td>
<td>cast</td>
<td></td>
</tr>
<tr>
<td>Kilkea 20</td>
<td>4</td>
<td>cast</td>
<td></td>
</tr>
<tr>
<td>Kilkea 20</td>
<td>16</td>
<td>cast</td>
<td></td>
</tr>
<tr>
<td>Leitrim 10</td>
<td>4</td>
<td>cast</td>
<td></td>
</tr>
<tr>
<td>Leitrim 10</td>
<td>16</td>
<td>cast</td>
<td></td>
</tr>
<tr>
<td>Leitrim 20</td>
<td>4</td>
<td>cast</td>
<td></td>
</tr>
<tr>
<td>Leitrim 20</td>
<td>16</td>
<td>cast</td>
<td></td>
</tr>
<tr>
<td>Kilkea 10</td>
<td>4</td>
<td>no cast</td>
<td></td>
</tr>
<tr>
<td>Kilkea 10</td>
<td>16</td>
<td>no cast</td>
<td></td>
</tr>
<tr>
<td>Kilkea 20</td>
<td>4</td>
<td>no cast</td>
<td></td>
</tr>
<tr>
<td>Kilkea 20</td>
<td>16</td>
<td>no cast</td>
<td></td>
</tr>
<tr>
<td>Leitrim 10</td>
<td>4</td>
<td>no cast</td>
<td></td>
</tr>
<tr>
<td>Leitrim 10</td>
<td>16</td>
<td>no cast</td>
<td></td>
</tr>
<tr>
<td>Leitrim 20</td>
<td>4</td>
<td>no cast</td>
<td></td>
</tr>
<tr>
<td>Leitrim 20</td>
<td>16</td>
<td>no cast</td>
<td></td>
</tr>
</tbody>
</table>
carbon in order to compensate for the fact that the amounts of organic matter varied between the soils.

3. Results

3.1. CO₂ production

Following the addition of water to the experimental units there was always an immediate and sharp increase in the amount of CO₂ evolved. In general, maximum CO₂ output occurred 2–4 h after the addition (Fig. 1). Following this peak there was a rapid decline for a period of 6–10 h followed by a slower decline over the remaining 3, or 15, d of the cycle.

Irrespective of treatment, the production of CO₂ was always greater at 20°C than at 10°C, with the production at 10°C being approximately 40% less. The

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Table 2
Table of F values for the response of carbon and nitrogen parameters to temperature (T), wetting cycle (W), soil (S) and cast (C) treatments.

*P < 0.05, **P < 0.01, ***P < 0.001

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d.f.</th>
<th>F values</th>
<th>d.f.</th>
<th>F values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td></td>
<td>water</td>
<td></td>
<td>DOC</td>
</tr>
<tr>
<td>S</td>
<td>1, 48</td>
<td>175.97***</td>
<td>1, 32</td>
<td>247.35***</td>
</tr>
<tr>
<td>W</td>
<td>1, 48</td>
<td>26.55***</td>
<td>1, 32</td>
<td>13.63***</td>
</tr>
<tr>
<td>T</td>
<td>1, 48</td>
<td>1076.09***</td>
<td>1, 32</td>
<td>8.53**</td>
</tr>
<tr>
<td>C</td>
<td>1, 48</td>
<td>1.81</td>
<td>1, 32</td>
<td>0.79</td>
</tr>
<tr>
<td>S x W</td>
<td>1, 48</td>
<td>72.89***</td>
<td>1, 32</td>
<td>10.17**</td>
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<tr>
<td>S x T</td>
<td>1, 48</td>
<td>31.07***</td>
<td>1, 32</td>
<td>9.14**</td>
</tr>
<tr>
<td>S x C</td>
<td>1, 48</td>
<td>0.15</td>
<td>1, 32</td>
<td>0.64</td>
</tr>
<tr>
<td>W x T</td>
<td>1, 48</td>
<td>1.2</td>
<td>1, 32</td>
<td>0.02</td>
</tr>
<tr>
<td>W x C</td>
<td>1, 48</td>
<td>2.24</td>
<td>1, 32</td>
<td>6.17*</td>
</tr>
<tr>
<td>T x C</td>
<td>1, 48</td>
<td>1.3</td>
<td>1, 32</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>S x W x T</td>
<td>1, 48</td>
<td>4.64*</td>
<td>1, 32</td>
<td>0.46</td>
</tr>
<tr>
<td>S x W x C</td>
<td>1, 48</td>
<td>2.46</td>
<td>1, 32</td>
<td>0.39</td>
</tr>
<tr>
<td>W x T x C</td>
<td>1, 48</td>
<td>4.27*</td>
<td>1, 32</td>
<td>0.08</td>
</tr>
<tr>
<td>S x T x C</td>
<td>1, 48</td>
<td>0.39</td>
<td>1, 32</td>
<td>1.27</td>
</tr>
</tbody>
</table>

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Fig. 1. Loss of CO₂ (µl CO₂ g⁻¹ soil C h⁻¹) from cast (—) and control ( - - - ) for Kilkea soil treatments during the 4 d wetting cycle. Bars represent the mean ± S.E.
production from the clayey Leitrim soil was 5–35% less than that from the Kilkea soil (Fig. 2). Although the interaction between wetting regime and temperature was not significant, there was a significant interaction between soil, wetting regime and temperature ($F_{v,3} = 19.27^{***}$) (Table 2). The response to temperature was most evident for the Kilkea soil under the 4 d cycle where the production of CO$_2$ was 1.85 times greater at 20°C. The increase for the Leitrim soil was only 1.6. However, this difference in response was not evident under the 16 d cycle where both soils showed an approximately 1.7-fold increase in production. Therefore it is apparent that soil type has a significant bearing on the relationship between C mineralization and temperature/moisture regime. It is also apparent that cast had no significant effects, but $Q_{10}$ values for cast treatments were lower than for the controls, suggesting that the cast aggregate structure limited de-
composition rates (at least at high temperatures) (Table 3). In the clayey Leitrim soil such differences were less and only present under the 4 d cycle, highlighting the overriding influence of clay content on microbial respiration.

3.2. Leachate volume

Several of the factors interacted significantly in

<table>
<thead>
<tr>
<th>Soil cycle</th>
<th>Kilkea</th>
<th>Leitrim</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 d</td>
<td>16 d</td>
</tr>
<tr>
<td>Cast</td>
<td>1.80</td>
<td>1.69</td>
</tr>
<tr>
<td>Control</td>
<td>1.89</td>
<td>1.74</td>
</tr>
<tr>
<td>Average</td>
<td>1.85</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Fig. 3. Cumulative volume (ml) of water leached from (a) Kilkea and (b) Leitrim treatments. Bars represent the mean ± S.E. Ec10 is earthworm cast at 10°C, Ec20 is earthworm cast at 20°C, Co10 is control at 10°C and Co20 is control at 20°C.
determining the volumes of leachate produced. Greater volumes (1–3 times) of water were leached from all systems under the 16 d cycle (Fig. 3). However, there were obviously smaller volumes of leachate at 20°C than at 10°C with the 4 d cycle, but this was not so obvious with the 16 d cycle \((F_{WT} = 534.96^{***})\). The soil type interacted with temperature and wetting regime in that there was a reduced volume of leachate for both soils at higher temperatures under the 4 d cycle but the volumes of leachate from the clayey Leitrim were not reduced at 20°C under the 16 d cycle. In other words the Kilkea soil retained more water than the Leitrim soil at the higher temperature when there was a significant period without water input, i.e. 16 d cycle. Although the interaction between cast and soil type was significant, its contribution to the variance was minimal.

3.3. Dissolved organic carbon

None of the third or fourth order interactions were significant for DOC. However, many of the factors
interacted in pairs. The effect of temperature and wetting regime differed between the soils. The average amounts leached from the Kilkea soil responded to the temperature and wetting factors, with more DOC being lost under the 16 d cycle and at 10°C ($F_{WT} = 61.23^{***}$). The Leitrim soil did not respond in a consistent manner to these factors (Fig. 4). Under the 4 d cycle both soils lost approximately twice as much DOC at 10°C than at 20°C. However, under the 16 d

Table 4
Ratio of CO$_2$–C-to-DOC from experimental treatments

<table>
<thead>
<tr>
<th>Soil cycle</th>
<th>Kilkea</th>
<th>Leitrim</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 d</td>
<td>16 d</td>
</tr>
<tr>
<td>Cast 10°C</td>
<td>15.81</td>
<td>8.74</td>
</tr>
<tr>
<td>Control 10°C</td>
<td>15.25</td>
<td>7.97</td>
</tr>
<tr>
<td>Cast 20°C</td>
<td>58.90</td>
<td>14.63</td>
</tr>
<tr>
<td>Control 20°C</td>
<td>67.23</td>
<td>15.17</td>
</tr>
</tbody>
</table>

Fig. 5. (a) Weighted average amounts of NO$_3$–N (ng NO$_3$–N g$^{-1}$ soil C) lost from (a) Kilkea and (b) Leitrim treatments. Bars represent the mean ± S.E. Ec10 is earthworm cast at 10°C, Ec20 is earthworm cast at 20°C, Co10 is control at 10°C and Co20 is control at 20°C.
cycle the Kilkea soil did not show a marked response to temperature while the response of the Leitrim soil varied between cast and noncast treatments.

The effect of the casts was to reduce the losses of DOC from the Leitrim soil. However, for the loamy Kilkea soil, as indicated above, the effect was dependent on the temperature. At 10°C less DOC was leached from the casts, but at 20°C there was more leachate.

3.4. Losses of carbon in CO₂ and DOC

In general, the ratios of CO₂–C/DOC losses from Leitrim soils were higher than from Kilkea soils under the 16 d cycle (Table 4). This was due mainly to considerably lower losses of DOC with this cycle. The reverse was evident under the 4 d cycle, though the differences were not as considerable.

The effect of the 16 d wetting/drying cycle relative
to the 4 d cycle was to reduce the CO$_2$–C-to-DOC ratio in the Kilkea soil. For the Leitrim soil the effect was more complex. At 20°C the 16 d wetting/drying cycle reduced the ratio, while the reverse was true at 10°C. Temperature had a considerable influence on carbon losses. With the 4 d cycle, the amount of C lost as DOC was less at 20°C than at 10°C. The reverse was true for the amount of C lost as CO$_2$. This resulted in a 4- and a 3-fold difference in the ratio of CO$_2$–C-to-DOC between temperatures for the Kilkea and Leitrim soils, respectively. A similar trend was evident with the 16 d cycle (both soils), though the difference was considerably lower, ranging between 5 and 40%, mainly due to higher losses of DOC with this cycle.

In general, cast treatments had lower DOC losses, and thus a higher CO$_2$–C-to-DOC ratio than control soils. The one exception was in the case of the Kilkea soil under the 4 d cycle at 20°C. In this case the opposite was true due to a combination of lower CO$_2$ and higher DOC losses. Differences in CO$_2$–C-to-DOC ratios between temperatures were reduced in cast treat-
ments (Kilkea soil) for both cycles. However, this was particularly obvious with the 4 d cycle. The same effect was evident with the 4 d cycle for Leitrim soils, but the reverse was true with the 16 d cycle. The percentage of the C loss in the form of DOC ranged between 1.7 and 15.5% for Kilkea soils and 2.3 and 6.9% for Leitrim soils.

Thus, wet/drying intensities, temperature, soil texture and cast structure all influenced the ratio of carbon lost in CO2 and DOC in this experiment.

3.5. Nitrate-N and ammonium-N

The amount of nitrate-N leached was dependent on complex interactions between all four experimental factors $F_{SWTC} = 4.27^*$. Under the 4 d cycle, amounts of nitrate-N in leachate from the Kilkea soil were between 40 and 60 times greater than those from the Leitrim soil (Fig. 5). The same trends were evident for the 16 d cycle but the sizes of the differences were less. In addition, more was leached under the 16 d cycle than under the 4 d cycle. The relationship between wetting cycle and cast varied between the soils ($F_{SWC} = 6.29^*$). With the Leitrim soil the response to casts was unaffected by wetting cycle, i.e. the amounts were generally higher coming from casts. This was also true for the Kilkea soil except when incubated at 20°C under the 4 d cycle, i.e. less nitrate was leached from casts produced from the loamy soil at 20°C under the 4 d cycle. The effect of temperature was very dependent on the other factors and while there may be a general pattern of increase at the higher temperature the opposite was true for the clayey Leitrim soil under the 16 d cycle where the amounts leached were reduced at 20°C.

The amount of ammonium-N leached was dependent on the soil type, the wetting regime and the interaction between them. In general, the amounts of ammonium-N in leachate from the Kilkea soil under both wetting regimes were more than 10 times greater than those from the Leitrim soil (Fig. 6). In addition, the amounts leached under the 16 d cycle were significantly larger than those under the 4 d cycle. While no significant effect of the casts was seen in the ammonium-N dynamics, several patterns do appear to emerge which are masked by the large within treatment variation. For example, more ammonium appears to be leached from the Kilkea soil casts, particularly under the 16 d cycle, while the opposite was true for the Leitrim soil.

Overall, treatments with the Kilkea soil lost approximately 25–35 times more total N than corresponding Leitrim soils under the 4 d cycle, and 10–20 times more under the 16 d cycle (Fig. 7). This illustrates the extent of nutrient protection in Leitrim soils, but also the reduction of this effect with super-saturation following prolonged drying (16 d cycle, 120 ml water application). Comparing wet/drying cycles, approximately one-third less total N was leached during the 4 d cycle than the 16 d cycle in Leitrim soils, while this difference was just 60% in the Kilkea soil.

4. Discussion

It is apparent from our study that temperature, frequency of wetting and drying, soil texture and cast aggregates influence the dynamics of C and N in soil. In addition, and perhaps more importantly, there are significant interactions between these factors.

4.1. Carbon loss

The production of CO2 was lower in the clayey soil, greater at 20°C for both soils, while the effect of wetting cycle was only apparent for the clayey soil. For both soils (and for both cycles) there was a rapid increase in CO2 production during the first 2–8 h after wetting followed by a decrease over the next 12–24 h of the cycle. Van Gestel et al. (1993) stated that after remoistening dried soils, available (labile) components are assimilated and transformed into new biomass and partly into CO2. Franzluebbers et al. (1994) found a similar pattern to CO2 losses, when plant matter, mixed with a loamy soil, was subjected to 5 d drying–wetting cycles. However, in their study the slower rates of change (both increases and decreases) may have been due to less water addition (relative to water holding capacity), while in our experiment, water addition was greater than water holding capacity for both cycles. This would have resulted in less disruption of organic–mineral bonds and, as a result, less nutrients would have been made available for microbial activity. De Bruin et al. (1989) found a more rapid decrease in CO2 production from their treatments by comparison with our results which may have been due to limited amounts of labile organic material in their treatments, since plant litter was not incorporated into their treatments. For the initial 2 months of our experiment CO2 output decreased slowly in all treatments as was the case in studies by De Bruin et al. (1989) and Franzluebbers et al. (1994) though in the latter, the rate of decrease in CO2 production on each successive cycle was considerably greater than in our experiment.

Van Gestel et al. (1993) stated then that the flush of C mineralization caused by drying/rewetting depends on the size of the soil organic pool, the quality of the organic matter, the extent to which it is protected by clay adsorption and on the properties of the soil biota. Certainly for the Leitrim soil, greater carbon stabilisation due to high silt/clay content (Mortland, 1986),...
may have lead to a reduction in CO₂ production in this soil (Jenkinson, 1971; Sorensen, 1972; Martin and Haider, 1986) and may have had an overriding influence on respiration rates. This idea is supported by the fact that more than 15% as much CO₂ was produced per g soil C from the Kilkea soil than from the Leitrim soil at both temperatures and in both cycles. The wetting cycles on the other hand dampened variations between soils (see Q₁₀ values, Table 3) while casts had no significant effect on respiration in our experiment.

Losses of DOC were not so influenced by temperature but the wetting cycle had a considerable influence in the loamy Kilkea soil. In general, slightly greater amounts of dissolved organic C were leached from uningested soil than from earthworm casts. These differences were particularly evident for the clayey Leitrim soil, where they occurred at both temperatures and both wetting/drying cycles. With the less clayey Kilkea the differences were not so obvious. During intense supersaturation of the soil, bonding may be weakened considerable by the replacement (during swelling) of cation bridges with weaker water bridges, a reversal of the process outlined by Shipitalo and Protz (1989) for cast formation. As a result, the differences between the strength of the cast and uningested soil aggregates (McKenzie and Dexter, 1987; Marinissen and Dexter, 1990) may be reduced or eliminated, resulting in losses of previously-protected organic matter and nutrients (Hindell et al., 1994b). Alternatively, moisture content below a critical minimum (after prolonged drying) may weaken the structural integrity (through denaturation of organic bonds, Hindell et al., 1997), to the point where differences between DOC losses in leachate from cast and control are eliminated (when rewetting occurs). It is interesting to note then, that during the initial phases (first three cycles) of the 16 d cycle (Kilkea soil), cast treatments leached less DOC than the control at 10°C, but on the fourth cycle the trend was reversed. At 20°C there were never any differences. This would suggest that the single water application (120 ml) had a more pronounced effect at 20°C, and that both supersaturation and drying intensity played a part in the disruption and dispersal of organic matter in cast aggregates.

The cumulative volumes of leachate were greater from the Leitrim soil for both cycles, indicating that the Kilkea soil retained more water than the Leitrim soil for these wetting/drying regimes. This in turn suggests that water bridges (during supersaturation) and potential swelling capacity may have had a greater influence in the Kilkea rather than in the Leitrim soils (the latter did not show a breakdown in aggregation).

Visually, it was clear that, in Leitrim soils, the cast macroaggregate structure maintained its integrity. A combination of organo–mineral bonding (Shipitalo and Protz, 1989) and inorganic cementing (Tisdall and Oades, 1982) may have increased cast aggregate strength to such an extent that the increased water application (120 ml) or increased intensity of drying (16 d cycle) failed to reduce the protection afforded to organic matter in the cast. Hindell et al. (1997) found that the dispersion index of air-dried casts was not significantly increased by 5 wet/drying cycles in a hard setting, silty loam soil. Van Gestel et al. (1991) stated that DOC variations between soil leachates were due to differences in CEC and microporosity (both of which are dependent or influenced by compaction and clay content) providing a level of protection during desiccation. Thus, overall concentrations and amounts of DOC in leachate from the Kilkea soil were 1–3 times greater than from the Leitrim soil.

The percentage of the C lost as DOC (1–15%) corresponds with other studies of C loss in forest systems. Bunnell et al. (1977) estimated, using modelled data from several countries, that losses due to microbial respiration are usually in the range of 70–90%, the rest being due to leaching of DOC and disintegration, while A.H. Magill (unpubl. Masters thesis, University of New Hampshire, 1996) monitored amounts of CO₂ and DOC in leachate from seven species of hardwood and softwoods and found that between 6 and 39% of total C loss was leached as DOC. In studies by Cronan (1985), 54–68% of the forest floor C loss was due to respiration, while DOC leaching accounted for the remaining 32–46% of C loss.

4.2. Nitrogen loss

Nitrogen (both nitrate-N and ammonium-N) losses in leachate from Kilkea soils under the 16 d cycle were higher from the casts than the control (at both temperatures, the difference being greater at the higher temperature). We can assume that initial pools of labile or mobile N were greater in the cast (Barley and Jennings, 1959; Haimi and Huhta, 1990; Scheu, 1993; Parkin and Berry, 1994). In addition, supersaturation (120 ml) may have reduced any protection or stabilisation effect inherently present in cast aggregates. Nitrate-N losses (from both cast and control) increased in the first month and then decreased, while other nutrients (e.g. ammonia) showed a steady decline. The increase can be attributed to nitrification of existing ammonium pools and/or greater mineralization of organic N. Jensen (1997) indicated that organic N could be assimilated directly by the microbial biomass without entering the soil inorganic N pool.

Nutrient losses under the 4 d cycle were not as consistent as under 16 d cycle. No differences were detected at 10°C but at 20°C more nitrate was leached from the control, perhaps indicating greater nitrification in this treatment. Alternatively, unlike the 16 d cycle (where breakdown in cast structure was impli-
...cated in nutrient release, see DOC section above), the cast structure during the 4 d cycle may have maintained greater protection/stabilisation of organic material and thus N mineralization would have been higher in the control.

For the Leitrim soils (both cycles), higher losses of chloride, sulphate (results not shown) and ammonium were evident in leachate from the control for the first 2–3 sampling dates. However, during the remainder of the experiment, this trend was either reversed or cast and control lost nutrients at the same rate. This suggests greater protection of nutrients in cast at first, followed by a reduction in protection (weakening of aggregate structure) in later cycles. The same is true of nitrate losses in the 16 d cycle, but not the 4 d cycle, where greater nitrification may have occurred in the cast than the control, a similar trend to that of seen in Kilkea soils with the 16 d cycle.

The influence of temperature on N transformations and specifically nitrification in forestry ecosystems has been reviewed extensively (Focht and Verstraete, 1977; Killham, 1990; Quemada and Cabrera, 1997; Carnol and Ineson, 1999). The latter study found that the temperature optimum for nitrate concentrations in leachate from undisturbed soil cores to be 11°C. However, it was pointed out that disturbed soils inevitably have increased nutrients and aeration. Increased nutrient availability can lead to higher nitrification optima (ranging between 20 and 40°C). Thus, nitrification (and associated leaching of nitrate) in our experiment is likely to have had an optimum closer to 20°C, which is consistent with leachate results from Kilkea soils in both cycles and in Leitrim soils during the 4 d cycle. However, for Leitrim soils with the 16 d cycle, higher clay content, in conjunction with greater ‘cementing’ stabilisation (Hindell et al., 1997) at 20°C, may have been the dominant influence on nitrifying bacteria, resulting in lower nitrate losses than at 10°C. This corresponds with the results of Couëtèaux and Sallih (1994) who found that the greatest sequestration of 15N was in soils with the highest cation exchange capacity, the highest clay content and the highest organic matter content.

In Leitrim soils the effect of cast structure on the leaching of total N was masked by the more influential effect of high clay content. However, in the Kilkea soils, the cast structure lost more total N than control soils during the 16 d cycle (after aggregate destabilisation), while the reverse occurred at 20°C during the 4 d cycle (due to less aggregate destabilisation).

5. Conclusion

The high levels of contingency in the outcomes of ecological interactions have recently been highlighted (Lawton, 1999; Bolger et al., in press). The large numbers of significant interactions, between all the factors examined in this experiment, provide further convincing evidence of such contingency.

The loamy Kilkea soil lost greater amounts of C and N than the clayey Leitrim soil per g soil C. Intense wetting cycles increased C losses (as DOC) from the loamy Kilkea soil, and C (as CO2 form) and total N losses from the Leitrim soil.

The effects of temperature and cast formation on nutrient losses were less clearcut and dependent on soil type and wetting cycle. Temperature increased CO2 losses and reduced DOC losses (during less intense wetting cycles), but had limited influence on total N losses. Time series results suggest implications for nutrient losses from casts in the field. With increasing intensity of wet/drying cycles, casts, it is predicted, will lose more nutrients (C, N) than uningested soils. But at higher temperatures (~20°C) with less intense wet/drying cycles, casts will retain more nutrients than uningested soils, particularly in soils which have a high silt/clay content. Thus, while both surface and buried cast material will reduce nutrient losses in leachate relative to uningested soil, losses are expected to be higher in surface cast, since surface material is exposed to wet/drying cycles of greater intensity than buried casts. To improve our understanding of SOM dynamics, cast mediated changes (i.e. increased organic–inorganic interaction and burial) need to be incorporated into future models of soil decomposition, perhaps by enhancement of the soil texture function along with litter incorporation.

Acknowledgements

The work was supported by the Environmental Research and Development Programme of the Commission of the European Union (Vamos Programme). We would like to thank Sylvia Dolan and Pat Neville for their technical help.

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