Impact of earthworm *Lumbricus terrestris* living sites on the greenhouse gas balance of no-tillage arable soil

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Abstract

We studied the effect of the deep-burrowing earthworm *Lumbricus terrestris* on the greenhouse gas (GHG) fluxes and global warming potential (GWP) of arable no-till soil using both field measurements and a controlled 15-week laboratory experiment. In the field, the emissions of nitrous oxide (N_2O) and carbon dioxide (CO_2) were on average 43 and 32% higher in areas occupied by *L. terrestris* (the presence judged by the surface midden) than in adjacent, unoccupied areas (with no midden). The fluxes of methane (CH_4) were variable and had no consistent difference between the midden and non-midden areas. Removing the midden did not affect soil N_2O and CO_2 emissions. The laboratory results were consistent with the field observations in that the emissions of N_2O and CO_2 were on average 27 and 13% higher in mesocosms with than without *L. terrestris*. Higher emissions of N_2O were most likely due to the higher content of mineral nitrogen and soil moisture under the middens, whereas *L. terrestris* respiration fully explained the observed increase of CO_2 emissions in the laboratory. In the field, the significantly elevated macrofaunal densities in the vicinity of middens likely contributed to the higher emissions from areas occupied by *L. terrestris*. The activity of *L. terrestris* increased the GWP of field and laboratory soil by 50 and 18%, but only 6 and 2% of this increase was due to the enhanced N_2O emission. Our results suggest that high N_2O emissions commonly observed in no-tillage soils can partly be explained by the abundance of *L. terrestris* under no-till management and that *L. terrestris* can markedly regulate the climatic effects of different cultivation practises.

1 Introduction

Agricultural soils can significantly contribute to the global greenhouse gas (GHG) exchange, but the contribution varies among the gases. For nitrous oxide (N_2O), the emissions from agricultural soils account for 60% of the anthropogenic emissions (Smith et al., 2007), whereas for methane (CH_4), mineral agricultural soils are usually sinks as the aerobic top soil favours methanotrophic bacteria (Hütsch, 2001). For carbon dioxide (CO_2), soils can be either sinks or sources depending on the balance of carbon input and output (Stockmann et al., 2013). N_2O emissions are mainly regulated by soil oxygen status, but also by the availability of nitrogen and organic carbon (Granli and Bøckman, 1994). The oxygen availability varies with soil structure and moisture and the potential for N_2O emissions is greatest when the water filled pore space (WFPS) is 60-70% (Davidson, 1991) as this enables both nitrification and denitrification. When the WFPS is above 70%, only denitrification takes place due to the shortage of oxygen and the dominating end product is the N_2 gas.

The application of no-tillage practice has recently increased in the agriculture (Derpsch et al., 2010). No-tillage often increases carbon sequestration to soils and is therefore considered as a useful cultivation technique in climate change mitigation (Lal, 1997). Elevated N_2O emissions may, however, decrease the atmospheric benefits of no-till (Li et al., 2005; Sheehy et al., 2013; Palm et al., 2014) as the denser physical structure (Tebrügge and Düring, 1999; Schjønning and Rasmussen, 2000) and higher moisture content (e.g. Sharratt, 1996; Gregorich et al., 2008) of no-tilled soils lead to higher N_2O emissions. The abundance and diversity of earthworms can also be markedly higher under no-till than conventional tillage (Edwards and Lofty, 1982; Chan , 2001; Rothwell et al., 2011) and the role of earthworms in the regulation and enhancement of GHG emissions has recently gained increasing attention. Field results are still scarce, but a recent meta-analysis of laboratory studies suggests that the presence of earthworms can increase N_2O and CO_2 emissions by 42 and 33%, respectively (Lubbers et al., 2013a). A number of factors potentially contribute to this phenomenon. For instance, by burrowing, casting and mixing crop residues into the soil, the earthworms change soil organic carbon cycling, porosity, aggregation and gas diffusivity, enhance decomposition and increase the amount of mineral nitrogen in the soil (e.g. Subler and Kirsch, 1998; Lubbers et al., 2011). Earthworm casts and burrow linings also have higher microbial activity and more denitrifying bacteria than the bulk soil (Svensson et al., 1986; Brown et al., 2000; Elliott et al., 1990) and the moist anaerobic environment in the earthworm gut can stimulate microbial N_2O production (Karsten and Drake, 1997; Drake and Horn, 2006). On the other hand, earthworms can

increase microaggregate formation and the stability of soil carbon (Fonte et al., 2007; Six and Paustian, 2014), and it is still unclear whether earthworms increase or decrease soil organic carbon stocks in the long term (Lubbers et al., 2013a; Blouin et al., 2013; Zhang et al., 2013).

Reduced tillage and no-till increase the densities of anecic, deep-burrowing earthworms in arable fields (Whalen and Fox, 2007). In the temperate and boreal fields, this group is mainly represented by the dew-worm, *Lumbricus terrestris* L. (Chan, 2001; Kladivko, 2001). In Finland, *L. terrestris* is the second most common earthworm species in arable fields, lagging only behind *Aporrectodea caliginosa* Sav. (Nieminen et al., 2011), and has the typical positive association with non-inversion cultivation (Nuutinen, 1992; Nuutinen et al., 2011). It is a large earthworm, which efficiently forages on crop residues (Subler and Kirsch, 1998; Shuster et al., 2000) and builds middens (i.e. small mounds of collected litter and surface castings) at the openings of its permanent burrows, often penetrating deeper than 1 m (e.g. Nuutinen and Butt, 2003). The middens are biological hotspots with high microbial activity (Schrader and Seibel, 2001; Aira et al. 2009), diverse invertebrate populations (Hamilton and Sillman, 1989; Maraun et al., 1999; Butt and Lowe, 2007) and higher nutrient and organic carbon contents than the surrounding soil (Subler and Kirsch, 1998; Wilcox et al., 2002; Aira et al., 2009). By transferring plant litter into the subsoil, *L. terrestris* may also increase the subsoil carbon stocks; e.g. Don et al. (2008) estimated that *L. terrestris* sequestrates carbon in the burrow linings at the rate of 22 g C m^{-2} yr^{-1}. On the other hand, the turnover time of burrow wall carbon can be only 3-5 years (Don et al. 2008). This is because the well aerated burrow walls allow the expansion of high microbial activity down the soil profile (Loquet et al., 1977 in Devliegher and Verstraete, 1997) and the interactions among microbes and their feeders in the burrow walls are intense and accelerate carbon and nutrient mineralization (Tiunov and Scheu, 1999; Görres et al., 1999; Görres et al., 2001). The burrows of *L. terrestris* are also bypass flow routes for percolating water, and depending on arable soil management they may increase leaching of topsoil nitrogen to the subsoil (Shuster et al., 2003).

Most of the investigations of earthworm effects on GHG emissions have been carried out in the laboratory (Bertora et al., 2007; Rizhiya et al., 2007; Giannopoulos et al., 2010; Lubbers et al., 2011; Augustenborg et al., 2012) and to our knowledge, only three field experiments have been conducted (Borken et al., 2000; Amador and Avizinis, 2013; Lubbers et al., 2013b). Recent reviews have underlined the need for field studies with all major gases (N_2O, CO_2 and CH_4) to provide a more comprehensive picture of earthworm contribution to soil GHG emissions (Lubbers et al., 2013a; Blouin et al., 2013). In this study, we aimed at filling this research gap by measuring the

small-scale spatial variation of soil biological and chemical properties and N_2O, CO_2 and CH_4 fluxes caused by *L. terrestris* in a northern, arable no-till field. We hypothesised that: (1) the N_2O and CO_2 emissions are greater on *L. terrestris* midden areas (higher earthworm activity) compared to adjacent non-midden areas (lower earthworm activity) while CH_4 emissions remain unaffected; (2) the middens contribute to gas production and their removal from soil surface decreases instant gas emissions; and (3) the biological and chemical soil properties essential for gas balance differ between the midden and non-midden areas. Moreover, to test how well the earthworm effects on GHG emissions in the field can be predicted by laboratory experiments, we established a controlled laboratory study with a *L. terrestris* treatment and measurements of response variables identical to those in the field. Our aim was not to establish a laboratory experiment that would perfectly mimic our field situation, but to establish a *typical* laboratory experiment to test whether laboratory studies in general can produce results that resemble the field results. This is an important aspect as most earlier experiments have been carried out in the laboratory and e.g. the review by Lubbers et al. (2013a) is entirely based on laboratory studies.

2 Methods

2.1 Field measurements

Field measurements of N_2O, CO_2 and CH_4 emissions were conducted in a long-term, no-till field (11 years of no-till cultivation) in Säkylä (60°58'N, 22°31'E), south-west Finland in October 2008. The soil at the site (depth 0-20 cm) is fine sand with 15% clay, 29% silt and 56% sand. Soil pH (H_2O) is 6.1 and the N and C concentrations 0.1 and 2.1%, respectively. The topsoil (0-5 cm) bulk density is 1.37 g cm^{-3}. The annual crops cultivated in the field in 2007 and 2008 were turnip rape and barley, respectively. Ten large middens and their adjacent non-midden areas were randomly chosen within two 20 m^2 areas (called sites A and B; five pairs in both) one month after crop harvest, which according to our experience is a time of high *L. terrestris* activity. The two sites, 30 m apart, were needed to obtain a sufficient number of treatment pairs, but they also provide data for testing whether the treatment effect varies in space at the field scale. For this purpose, the site was included in the statistical models as an explaining factor. In order to minimize the environmental variation within treatment pairs, the distance between the midden and non-midden areas within a pair was kept short; the average distance between the outer rims of measurement chambers within a pair was 13 cm (min 3 cm, max 34 cm), while the average distance between a pair and its closest counterpart was 1.35 m (min 0.37 m, max 3.00 m).

The gas measurements were accomplished using round PVC chambers (diameter 15 cm, height 10 cm). Five gas measurements were carried out at varying intervals over a period of two weeks. Chambers were pressed into the soil to the depth of approximately 2 cm and the soil was compressed by hand around the chambers. Permanent installations were not established in order to avoid the disturbance of earthworms, and since the experiment was conducted after harvest, it was not necessary to take into account the decrease of CO_2 flux that may follow when live roots are cut by the chamber (see Heinemeyer et al. 2011). In each measurement, 20 ml of chamber air was sampled through a rubber septum using a polypropylene syringe (BD Plastipak, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) immediately and 60 min after the placement of the chamber. The air was then transferred into pre-evacuated 12 ml glass vials (Exetainer, Labco Ltd., High Wycombe, UK). Before each gas sample, the air in the chamber was mixed by one syringe flush.

The air temperature, which was measured using a Fluke 52 II thermometer (Fluke Corp., USA), fluctuated between 7.2 and 11.8 ºC during the gas measurements. Air temperature, instead of the chamber temperature, was used to define the gas volume for flux calculation as chamber warming due to radiation is minimal in October. Soil moisture was measured next to each 'midden – non-midden' pair at the depth of 0-15 cm during each gas measurement using a TRASE system I moisture meter and Time Domain Reflectometry (TDR) (Soil Moisture Equipment Corp., Goleta, CA, USA). The changes in soil temperature were followed using thermologgers (ElcoLog, Elcoplast Oy, Finland), which were installed at the depth of 5 cm outside the gas sampling areas (this data is missing for the two first gas measurements).

At the last measurement, gas samples were first taken as described above. The middens (surface cast mounds and the associated residues) and the straw litter of the non-midden areas were then removed and the gas measurements were repeated to evaluate the effect of midden and straw material on gas emissions. After these measurements, soil cores (diameter 5 cm, depth 5 cm) were collected from the entrance of *L. terrestris* burrows and the adjacent non-midden-areas. The removed midden and straw material and the soil samples were stored at -18°C for 7.5 months before analysed for gravimetric moisture content, potential denitrification and mineral N concentrations. To estimate earthworm abundances at the area of the gas measurement, the measurement chamber was pushed deeper to the soil and the earthworms were hand-sorted out of the obtained soil sample (diameter 15 cm, depth 15 cm). Deep-residing earthworms were extracted from the bottom of the pit by pouring 0.5-0.75 l formalin solution (0.5%) to the pit and collecting individuals that emerged

within 30 min. Slugs, which were abundant in the middens, were hand-sorted from the midden and non-midden area samples and together with the earthworms stored in 85% ethanol, weighted and identified into the species or genus level (Sims and Gerard, 1999; Kerney and Cameron, 1979).

2.2 Laboratory experiment

The soil, barley stubble straw and *L. terrestris* individuals were collected for the laboratory mesocosms in the beginning of November 2008 from the same no-till field that was used for field measurements. The 15-week experiment was designed to simulate the post-harvest autumn conditions of a no-till field and during the set-up, all unnecessary manipulation of soil, straw and earthworms was avoided to preserve the natural communities of microbes and soil micro- and mesofauna. The moist soil (moisture content 27% of fresh mass) was first sieved (6 mm) and mixed to ensure soil homogeneity. Any earthworms found were removed. Thirty PVC-tubes (diameter 15 cm, height 45 cm, bottoms enclosed with plastic lids) were then filled with the soil to the height of 43 cm. During filling, the soil was compacted to achieve even bulk density among the tubes (mean 1.43 g cm^{-3}, min 1.40 and max 1.46 g cm^{-3}, n=30). The tubes were weighted (before and after filling) and placed in an incubation room at 15-17°C, chosen as favourable temperature for *L. terrestris* activity (Butt, 1991), with a rhythm of 10 h day (fluorescent lamps providing on average 1102 lx) and 14 h night (no illumination). Air humidity was maintained using a moistener, but varied from 26 to 81% during the experiment. Soil moisture content was adjusted to 28% and kept approximately constant by adding deionized water once a week (always 2 d before gas samplings) and spraying the soil surface with water after gas measurements.

The *L. terrestris* individuals used in the experiment were extracted from the field using a mustard mixture (Gunn, 1992) and immediately washed in tap water. Individuals were kept in moist soil for 9 d (dark, 4°C) before one large individual was added to each of the 15 randomly chosen mesocosms. Each individual was weighted (mean fresh mass 4.5 g, min 3.7 g, max 5.5 g) and the settling into the soil was facilitated by creating an artificial burrow (depth 8.5 cm, diameter 0.5 cm) in the centre of the soil column. The remaining 15 mesocosms were left without worms and served as controls. The *L. terrestris* and control mesocosms were randomly placed in the incubation room as treatment pairs. An even layer of chopped straw was added on the top of the soil in each mesocosm (straw length 2 cm, total fresh mass 5 g), and to prevent animal escape, the mesocosms were covered by a mesh. Emerging plant seedlings were removed from the mesocosms during the experiment, whereas juvenile earthworms, noticed to hatch from the cocoons, were not as the removal would have disturbed the experiment.

The gas measurements were started one month after mesocosm establishment and were repeated twelve times, at one week intervals, from December 2008 to February 2009. The sampling was always carried out within one day. For the measurements, airproof plastic lids (diameter 15 cm, height 10 cm) were first placed on the tubes air-tightly. The incubation lasted for 60 min and the samples were collected according to the field protocol described above. At the final date, gas fluxes were measured before and after removing *L. terrestris* midden and straw residues. The soil samples for soil moisture, potential denitrification and mineral N measurements were taken as in the field. The tubes were emptied and the *L. terrestris* individuals and earthworm juveniles, hatched from the cocoons during the experiment, were hand sorted out of the soil. A 100 g subsample was taken from the mixed soil to estimate the mineral N content of the entire soil column. At the end of the experiment, three of the *L. terrestris* mesocosms had 1-3 and seven of the control mesocosms 1-2 small earthworm juveniles (both dark and light pigmented unidentified species) having a maximum individual fresh mass of 0.16 g. All earthworms were washed in deionized water and weighted and in order to determine their GHG production, incubated in 210 ml flasks for 60 min (separately for experimental *L. terrestris* and the group of juveniles). The GHG production was estimated using 10 ml gas samples taken in the beginning and at the end of the incubation. Three incubations of *L. terrestris* produced deviant fluxes of N_2O, CO_2 and CH_4 and the results were excluded from the data set.

2.3 Analyses of gases, potential denitrification and mineral nitrogen

The gas samples were always analysed within 48 h after sampling using a gas chromatograph (GC) equipped with a flame ionizer (FID), an electron capture detector (ECD) and a nickel catalyst for converting CO_2 to CH_4. The precolumn and analytical columns consisted of 1.8 and 3 m long steel columns, respectively, packed with 80/100 mesh Hayesep Q (Supelco Inc., Bellefonte, PA, USA). The GC (HP 6890 Series, GC System, Hewlett Packard, USA) had a 10-way valve with a 2 ml sample loop and a backflush system for separating water from the sample and for flushing the precolumn between the runs. A six-way valve was used to lead the flow to either the FID or ECD. The temperature of the GC oven, FID and ECD was 70 °C, 300 °C and 350 °C, respectively. Nitrogen was used as the carrier gas and a mixture of argon and methane (5%) as a make-up gas (1.4 ml min^{-1}) to increase the ECD sensitivity. A standard gas mixture (AGA Gas AB, Lidingö, Sweden) of known N_2O, CH_4 and CO_2 concentrations was used for the calibration curve. The flux rate of each gas was calculated using the gas accumulation rate during the 60 min enclosure period. Cumulative fluxes were calculated by assuming linear changes between subsequent measurement

dates. The net gas balance as a global warming potential (GWP) was determined using the factor 298 for N_2O and 25 for CH_4 (Myhre et al., 2013).

The denitrification potentials of the midden soil and the straw of the *L. terrestris* middens and the adjacent non-midden areas was determined as in Klemedtsson et al. (1988) and Henault et al. (1998) with some modifications. In brief, the defrosted and sieved 10 g (d.m.) soil samples (moisture was on average 26% in the field and 21% in the laboratory samples) were placed in 120 ml bottles and 4 ml of distilled water was added. The straw samples were combined within treatments (midden vs. non-midden, separately for areas A and B), because the amount of material in one sample was not enough for the analysis, and then divided to 2.5-5.5 g (d.m.) subsamples. After one night at 6 °C, the samples were transferred to 25°C and treated with 5 ml of potassium nitrate (KNO_3) solution and 5 ml of glucose solution (corresponding to amendments of 200 mg N and 500 mg C kg^{-1} soil). The bottles were then sealed using butyl rubber septa and crimp seals, evacuated and flushed three times with dinitrogen gas. The overpressure in the bottles was released through a 0.5 mm needle, pierced through the septum, and to prevent the entry of oxygen into the bottle, the needle was mounted on a 1 ml plastic syringe (without piston) filled with 0.1 ml distilled water. The bottles were then amended with 12 ml of acetylene (C_2H_2) to block the N_2O reduction step of denitrification, which was regarded as the start of the incubation (t=0). Three-ml gas samples were then taken after 15 and 45 minutes, followed by one-ml samples after 75, 105, 135, 165, 195, 225 and 255 minutes and these were injected into 12-ml evacuated vials. All samples were diluted with N_2 to a volume of 18 ml to ensure that the concentrations were in the range of the calibration curve. Samples were analyzed using the Hewlett Packard GC as described above.

For the analyses of soil ammonium and nitrate concentrations, samples were first homogenized manually using a steel spatula, and from each sample 50 g of fresh soil was mixed with 125 ml of 2 M KCl and shaken for 2 hours on an orbital shaker. The amount of straw material in one sample was too small for the analysis, so straw samples were combined within treatments. The combined samples were then divided to 6-21 g (fw) subsamples and treated similarly as the soil samples. The extracts of soil and straw samples were filtered through filter paper (130 g m^{-2}, Tervakoski Oy, Tervakoski, Finland) and analysed for nitrate and ammonium the next day after storage at 6 °C. A colorimetric autoanalyser (QuikChem AE, Lachat Instruments, Loveland, Colo., USA) was used for the simultaneous analysis of nitrate and ammonium.

2.4 Statistical analyses

The field data of N_2O, CO_2 and CH_4 emissions were obtained from a randomized complete block design with repeated measurements. Altogether, there were ten pairs (blocking factor) of midden - non-midden areas (treatment factor) from the two sites (A and B). The measurements at the same experimental site were correlated, which was taken into account in the statistical models through appropriate covariance structures. The statistical model thus became:

$$y_{ijkl} = \mu + s_i + \beta_{j(i)} + t_k + (st)_{ik} + \varepsilon_{ijk} + d_l + (sd)_{il} + (\beta d)_{jl(i)} + (td)_{kl} + (std)_{ikl} + \gamma_{ijkl} \quad (1)$$

where μ is constant intercept, s_i, t_k, $(st)_{ik}$, d_l, $(sd)_{il}$, $(td)_{kl}$ and $(std)_{ikl}$ are fixed main and interaction effects for site (s), treatment (t) and date (d). The $\beta_{j(i)}$ is the random effect for block j within site i and ε_{ijk} is random plot to plot variation, all mutually independent with variances var($\beta_{j(i)}$)=$\sigma 2_{\beta}$, and var(ε_{ijk})= $\sigma 2_{\varepsilon}$. The $(\beta d)_{jl(i)}$ represents the random date-specific contribution for block i within site j, and γ_{ijkl} represents random error effect for observations on the same plot (Gumpertz and Brownie, 1993). This model was used for CH_4. For N_2O and CO_2, a simplified model was used as the site had no effect on the fluxes of either gas. The effect of removing middens and straw litter from the soil surface on N_2O, CO_2 and CH_4 emissions was analysed using similar model as for the repeated gas measurements, except that the repeated measurement effect of date was replaced with the repeated measurement effect of before and after removal. Analogously to the earlier models, the site effect was included in the model for CH_4, but not for N_2O and CO_2. In the case of N_2O, log-transformation was used to meet the normality assumption.

The background variables were measured at the last measurement date (Table 4). Since these measurements were not repeated, the statistical models used were simplified analogues of the model presented above, except for the number of slugs, which was analysed using the non-parametric Wilcoxon Sign Rank test as the assumptions of the parametric methods were not met. The cumulative emissions of N_2O, CO_2 and CH_4 were analysed using a simplified non-repeated analogue of the model presented above. The analysis of laboratory data followed the analysis of field data, except that the site effect and interactions were not included in the models. Log-transformations were used for N_2O and mineral nitrogen (top 5 cm soil samples) and in addition, two outliers were excluded from the mineral nitrogen data due to exceptionally high values in comparison to the other 13 observations in the control mesocosms.

For all the parametric models, REML was used as the estimation method, degrees of freedom were calculated by the Kenward-Roger method (Kenward and Roger, 1997) and model assumptions were

checked using appropriate graphs. The models were fitted using the MIXED procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and pairwise comparisons were performed using two-sided *t*-type tests.

3 Results

3.1 Field measurements

In the field, the N_2O and CO_2 emissions were significantly higher in the midden than non-midden areas (Table 1; Fig. 1a, b). The overall (all repeated measurements included) model-based mean estimates of N_2O fluxes were 0.23 (95% CI 0.18-0.27) and 0.13 (0.09-0.17) µg N chamber area^{-1} h^{-1} for the midden and non-midden areas, respectively. The corresponding figures for CO_2 were 1754 (1568-1941) and 1201 (1015-1388) µg CO_2 chamber area^{-1} h^{-1}, respectively. Based on these estimates, the chamber area with one midden produced on average 43% more N_2O and 32% more CO_2 than an equivalent non-midden area. N_2O and CO_2 emissions varied among the dates (Fig. 1a, b; Table 1), but this variation was apparently not explained by soil moisture or temperature, which fluctuated little among the dates (min-max 37.2-38.3% and 6.5-8.5 ^{o}C, respectively). The CH_4 fluxes differed between the midden and non-midden areas at two measurement dates, but the effects were specific to the measurement site (Table 1): i.e. the flux was higher in the midden than non-midden areas in site B at the first measurement ($t_{14.1}$=-4.02, p=0.001), but lower in site A at the fourth measurement ($t_{12.4}$=2.44, p=0.031) (Fig. 1c, d). The model-based mean estimates of cumulative emissions were significantly higher in the midden than non-midden areas for N_2O and CO_2 ($F_{1,7.34}$=16.91, p=0.004; $F_{1,7.66}$=43.80, p<0.001, respectively), but not for CH_4 ($F_{1,7.74}$=3.24, p=0.111) (Table 2). The removal of middens and other residues from the soil surface had no effect on N_2O and CO_2 emissions in either the midden or non-midden areas (Table 3; Fig. 1a, b). For CH_4, the removal decreased the flux in site A ($t_{9.1}$= 2.86, p=0.019), but not in site B ($t_{7.87}$= -0.65, p=0.532) and no difference was found between the responses of midden and non-midden areas (Table 3, Fig. 1c, d).

The number of earthworms was 125% and their biomass 150% higher in the midden than non-midden areas (Table 4). However, only in four midden and two non-midden areas, a large (> 0.8 g) *L. terrestris* was found and the majority of earthworms were juveniles. In the midden areas, 18% of individuals belonged to *Lumbricus*, 51% to *Aporrectodea* and 31% remained unidentified. In the non-midden areas, the corresponding figures were 16%, 58% and 26%, respectively. The soil

surrounding the burrow entrance (within 5 cm diameter) was on average 1% unit moister, contained 23% more mineral N and had 20% higher potential denitrification than the top soil of the non-midden areas (Table 4), but the denitrification potential of the midden and non-midden straw did not differ (2.7 vs. 2.8 μg N_2O-N g^{-1} straw d.m. h^{-1}, respectively). The mineral N content of the straw was 28 and 69 mg kg^{-1} straw d.m. in the midden and non-midden areas, respectively, while the midden areas had more straw litter on the soil surface (visual observation). In total, 31 slugs (*Arion fasciatus* N.) were found from the midden areas after the final gas measurement, while only three were found from the non-midden areas (Table 4). In the midden areas, 77% of the slugs were found in the midden, 23% in the soil beneath the midden.

3.2 Laboratory experiment

In the laboratory, N_2O and CO_2 emissions were significantly higher with than without *L. terrestris* (Table 1; Fig. 2a, b). The model-based mean estimates (with all repeated measurements included) of N_2O emissions with and without *L. terrestris* were 0.060 (95% CI 0.053-0.067) and 0.044 (0.039-0.049) μg N chamber base $area^{-1}$ h^{-1}. The corresponding figures for CO_2 flux were 1769 (1600-1937) and 1536 (1367-1704) μg CO_2 chamber base $area^{-1}$ h^{-1}, respectively. Based on these values, one *L. terrestris* individual increased the mesocosm emission of N_2O and CO_2 by 27 and 13%, respectively. On average, the fluxes of N_2O and CO_2 decreased in the course of the experiment (Fig. 2a, b). The CH_4 flux fluctuated during the experiment without a clear trend (Table 1b, Fig. 2c) and only at day 98, the emission rate differed between the treatments, being then higher with than without *L. terrestris* (t_{171}= -2.12, p=0.035). The model-based mean estimates of the cumulative emissions were significantly higher with than without *L. terrestris* for N_2O and CO_2 ($F_{1,12.9}$=5.09, p=0.042; $F_{1,9.65}$=29.21, p<0.001, respectively), but not for CH_4 ($F_{1,11.5}$=0.33, p=0.579) (Table 2).

The removal of middens and straw residues from the soil surface affected the N_2O and CO_2 emissions, but not the CH_4 emissions (Table 3; Fig. 2a-c). The N_2O emissions increased after the removal in all mesocosms, whereas the response of CO_2 flux depended on the treatment: the removal increased CO_2 emissions in the presence (t_{26}= -3.36, p=0.002), but had no effect in the absence of *L. terrestris* (t_{26}= -0.64, p=0.525).

At the end of the experiment, mesocosms with *L. terrestris* had less straw litter on the soil surface (visual observation) and 4% more mineral N in the 0-43 cm soil column (excluding the soil core collected around the burrow) than the mesocosms without *L. terrestris* (Table 5). In all except two mesocosms the resident worm had created a burrow that reached the bottom of the soil column. The

soil that surrounded the *L. terrestris* burrow entrance (diameter 5 cm) was 0.3% unit moister, contained 16% more mineral N and had 17% greater potential denitrification rate than the top soil of the control treatment (Table 5). The potential denitrification of the straw collected from *L. terrestris* and control mesocosms was 0.24 and 0.19 µg N_2O-N g^{-1} straw d.m. h^{-1} and its mineral N content 664 and 122 mg kg^{-1} d.m., respectively.

Two of the 15 *L. terrestris* individuals had died and the rest 13 had lost on average 1.0 g or 22% weight during the 15-week experiment. When incubated in glass flasks at the end of the experiment, the mean emission rate of one *L. terrestris* individual (mean fresh mass 3.6 g, min 3.1 g and max 4.2 g) was 0.006 (SE 0.001) µg N_2O-N, 425 (41) µg CO_2 and -0.001 (0.002) µg CH_4 h^{-1}. Mean emissions per unit fresh mass (min, max) for the three gases were 0.06 (0.03, 0.12), 2678 (1501, 4197) and -0.03 (-0.19, 0.12) nmol gas g^{-1} f.w. h^{-1}, respectively. Based on these values, the proportion emitted by *L. terrestris* of the total N_2O, CO_2 and CH_4 fluxes at the last gas measurement was 16, 36 and 0.7%, respectively.

4 Discussion

In agreement with our first hypothesis, field N_2O and CO_2 emissions were greater in *L. terrestris* midden than non-midden areas. CH_4 fluxes were variable without a clear effect, but there was a slight indication that the presence of *L. terrestris* decreased the CH_4 oxidation rate of the soil. Against our second hypothesis, the removal of middens and residues from the soil surface did not decrease N_2O and CO_2 emissions. This indicates that the effect of *L. terrestris* on GHG emissions results from changes in soil conditions at its living site, not from the surface midden. Following our third hypothesis, most of the investigated biological, chemical and physical soil variables differed between the midden and non-midden areas, telling of the significance of *L. terrestris* as an ecosystem engineer in arable fields. The fact that we found equally positive effect of *L. terrestris* on N_2O and CO_2 emissions in the laboratory further indicates that the observed effects in the field cannot be purely explained by confounding factors such as the burrows acting as a chimney for gas emissions from a larger area than the chamber, the worms selecting sites of high microbial activity, or *L. terrestris* affecting the emissions of the adjacent control area by collecting straw from it. However, the magnitude of the effect was significantly smaller in the laboratory than in the field, i.e. 27% vs. 43% increase for N_2O and 13% vs. 32% increase for CO_2. It also appeared that the laboratory test could not fully simulate the role of *L. terrestris* middens in gas emissions as the

removal of middens increased the emissions. These results underline the value of comparing the measurements in laboratory to those in natural field sites with established earthworm populations.

Our results show that *L. terrestris* can create sites of elevated N_2O emissions in arable no-till soils: in the field, the cumulative N_2O emissions were 36% higher in the midden than non-midden areas and in the laboratory, 19% higher in mesocosms with than without *L. terrestris*. These results are in good agreement with earlier laboratory studies (e.g. Matthies et al. 1999; Giannopoulos et al., 2010), but also with field studies, such as the study by Borken et al. (2000), which reported a 57% increase in N_2O emissions in beech forest mesocosms due to *L. terrestris*. The recent meta-analysis of laboratory studies by Lubbers et al. (2013a) also suggested a 42% increase in soil N_2O emissions in the presence of earthworms. Few opposite findings exist (e.g. Speratti and Whalen, 2008) although some studies suggest that the contribution of earthworms to N_2O emissions could be transient (Amador and Avizinis, 2013; Lubbers et al., 2013b). In general, the contribution of earthworms to GHG emissions is composed of direct and indirect emissions. Direct emissions originate from earthworm metabolism and indirect from those changes the earthworms induce in their environment. Living earthworms have been found to emit N_2O (Drake et al., 2006; Karsten and Drake, 1997) and our incubation measurements support these findings (Table 6). The reported values of direct N_2O emissions emitted by *L. terrestris* vary from 0.05 to 0.95 nmol N_2O-N g^{-1} f.w. h^{-1} (Matthies et al., 1999; Horn et al., 2006; Wüst et al., 2009), so our value, 0.06 nmol of N_2O-N g^{-1} f.w. h^{-1} is at the lower end of this range.

Although the direct N_2O emissions have been quantified in many studies, there are few estimations of their proportion of total emissions. In our laboratory experiment, the proportion emitted by *L. terrestris* of the total N_2O flux was on average 16%, which is in good agreement with that reported by Karsten and Drake (1997) for beech forest soil (16%), but significantly higher than their value for oak-beech forest soil (0.25%). Our estimate is high and it may overestimate the proportion in the field because the time interval *L. terrestris* was able to shape the soil was short in our laboratory trial. In the field, the soil is subjected to a long-term earthworm impact and it is likely that this leads to a greater contribution of indirect emissions from the environment. It should also be noted that part of the N_2O produced by the earthworms may be reduced to N_2 while diffusing from the soil to the atmosphere and the significance of direct emissions may also for this reason in the field be lower than estimated based on laboratory measurements. Consequently, it is likely that the enhanced N_2O emissions in the presence of *L. terrestris* are also due to the changes in topsoil conditions and creation of hot spots of high biological activity, including the elevated macrofaunal

densities, in the vicinity of the middens. For instance, the higher content of mineral nitrogen and soil moisture favour denitrification, which was manifested as elevated values of potential denitrification in our measurements. In our field site, soil moisture was nearly 40%, corresponding to 80% WFPS, which is suitable for earthworm N_2O contribution (Evers et al., 2010). Another potential mechanism for increased N_2O emissions in the field are the burrows that may act as large pores that ease the diffusion of N_2O from the bottom soil and allow more of the N_2O ending up in the atmosphere without being reduced to N_2. The laboratory soil was dryer than the field soil, which could be one reason for the less noteworthy earthworm effect as soil moisture can significantly modify the earthworm-induced N_2O emissions (Chen et al. 2014).

The increase in soil cumulative CO_2 emissions due to the presence of *L. terrestris* was 33% and 15% in our field and laboratory measurements, respectively. These results echo the meta-analysis by Lubbers et al. (2013a), which suggests a 33% increase in soil CO_2 emissions in the presence of earthworms. When we estimated the respiration of individual earthworms in the laboratory, the mean CO_2 emission (425 μg h^{-1}) was almost double to the mean difference between the mesocosms with and without *L. terrestris* (230 μg chamber area h^{-1}) and the proportion of the total CO_2 flux explained by earthworm respiration was 36%. These values suggest that the increased emissions of CO_2 from the soils occupied by *L. terrestris* were fully explainable by the respiration of the animal itself. If this is true in general, the discrepancy between the observations of increased CO_2 emissions vs. increased carbon stability (Lubbers et al. 2013a) would be explained by earthworm respiration counteracting the enhanced carbon sequestration. However, this conclusion has to be treated cautiously as we do not know how well the measurements of earthworm respiration in the laboratory represent the respiration in the field. In the field, the elevated slug densities of the middens also likely contributed to increased CO_2 emissions as snail castings and mucus have been observed to increase the efflux from leaf litter (Theenhaus and Scheu, 1996). Snail activity accelerates N cycling, too (Theenhaus and Scheu, 1996), but we are not aware of any studies of snail impacts on N_2O emissions.

Unlike the effects on N_2O and CO_2 fluxes, the effects of *L. terrestris* on CH_4 flux were variable and mostly inconsequential and there was only a slight indication in the cumulative field fluxes that the presence of *L. terrestris* might decrease soil CH_4 oxidation rate. Such a decrease could be a consequence of increased moisture and N content in the vicinity of middens (Hütsch, 2001). Small and varying earthworm effects on net CH_4 fluxes have also been reported earlier (Borken et al., 2000; Aira et al., 2009; Bradley et al., 2012), and our estimate of 0.7% *L. terrestris* contribution to

the total CH_4 flux is in good agreement with the earlier statement that *L. terrestris* is not a source of CH_4 (Šustr and Šimek 2009). As CH_4 fluxes are also in general non-significant in the context of carbon cycling in boreal arable soils (Regina et al., 2007), it appears that the effects of earthworms on the GWP of these soils are driven by their effects on N_2O and CO_2 emissions.

Recent studies suggest that Finnish no-till fields are characterised by both high population densities of *L. terrestris* (Nuutinen et al., 2011) and elevated N_2O emissions (Sheehy et al. 2013). Higher N_2O emissions are usually explained by denser soil structure and higher soil moisture compared to tilled soils. Our results suggest that increased population densities of *L. terrestris* can also contribute to the elevated N_2O emissions. We found on average 20 *L. terrestris* middens per m^2 in our no-till field and when compared to a square meter of equal field with no middens, such a density would increase the N_2O emissions by 27% (estimated using mean values of midden and non-midden areas). Although this estimate has to be treated with caution as the non-midden areas were not completely out of the reach of *L. terrestris* activity, it appears that enhanced earthworm activity may explain a substantial part of the 60-150% increase in N_2O emissions observed in Finnish no-till fields (Sheehy et al. 2013). Moreover, when all three gases were considered together, *L. terrestris* increased the GWP of the soil by 50% and 18% in our field and laboratory investigations, respectively. These values, and particularly the field estimate, exceed the 16% mean increase in the net GWP of laboratory soils reported by Lubbers et al. (2013a) in their meta-analysis based on 33 observations from individual earthworm studies that reported the cumulative emissions of both N_2O and CO_2. However, the temporal variation in emissions is probably high, mainly due to soil moisture variation. For example, in a field study by Lubbers et al. (2013b), earthworms increased N_2O emissions of managed grassland in the autumn when the WFPS of soil was 61-65%, but had no effect in the dry spring when the WFPS was 16-25%. Our field experiment represents the conditions that prevail for approximately three months in the autumn when *L. terrestris* is highly active and it is possible that during other seasons, the gas emissions are less affected by the species. Moreover, the field estimate may exaggerate the earthworm effect as part of the straw in the non-midden areas was likely transferred and consumed in the midden area. In contrast to what we expected, the contributions of earthworm-induced N_2O and CO_2 emissions to the net increase in GWP were 6% and 94% in the field and 2% and 98% in the laboratory, respectively. This indicates that the elevated N_2O actually has a minor significance in the total balance despite its high GWP value.

One of our aims was to test whether the earthworm effects on GHG emissions that are found in laboratory trials can be generalized to field conditions. For this purpose, we established a mesocosm experiment using soil and *L. terrestris* individuals collected from the field site. The mesocosms had generally higher CO_2 and lower N_2O emission rates than the field soil, which probably was due to soil sieving increasing the availability of microbial resources and microbial respiration (Hartley et al. 2007) and drier mesocosm soil supporting lower N_2O production. Unlike in the field, the flux rates also steadily decreased in the laboratory, which probably tells of diminishing resource availability after the initial resource pulse (Hartley et al. 2007). Despite these differences in the level and dynamics of the flux rates, a clear, positive effect of *L. terrestris* on N_2O and CO_2 emissions was found in both systems. The magnitude of *L. terrestris* effect was smaller in the laboratory, which could be related to soil moisture and the loss of earthworm weight over the experiment, but also to the significantly elevated faunal abundance and activity in the long-lived *L. terrestris* living sites in the field. The size of the effect on CO_2 emissions also decreased in the laboratory as the experiment proceeded. Such a decrease is common in laboratory studies (Borken et al., 2000; Lubbers et al., 2013a) and is most probably related to the lack of fresh plant input to the soil, which has a negative impact on *L. terrestris* metabolism. The distinct difference between the field and laboratory emissions in their response to the removal of middens and residues from the soil surface is possibly to be explained by the lack of air current in laboratory conditions, which may have led to GHG accumulation in the soil pores and release of gases when the midden and straw were removed. All these findings suggest that while the general influence of *L. terrestris* on GHG emissions can be approximated in laboratory conditions, field measurements are needed for more accurate estimates and proper mechanistic understanding.

To conclude, our study contributes to filling the gap of field studies of the effects of earthworms on GHG emissions, particularly in soils long occupied by earthworms (Lubbers et al., 2013a). Our results emphasize the significance of *L. terrestris* in the gas balance of agricultural soils, and especially in no-till fields. We showed that *L. terrestris* respiration can explain the observed increase in CO_2 emissions in the presence of earthworms and that a substantial part of the increase of N_2O emissions in no-till arable lands can be explained by earthworm contribution. The gap of knowledge that still remains after our study is that the effects of earthworms have almost solely been studied in the absence of plants and without considering plant growth. As the effects of earthworms on plant growth are generally positive (van Groenigen et al., 2014), the dis-service of increased N_2O emissions may be counteracted by enhanced plant growth to the degree that no increase in yield-scaled emissions results (Wu et al., 2015). Extrapolation from our results to field

scale may neither be simple as the effect of midden density on GHG production is not necessarily linear due to resource competition among earthworm individuals. However, considering that field soils with active *L. terrestris* middens had 50% higher global warming potential than non-midden areas, it is clear that *L. terrestris*, and potentially other earthworm species as well, are among the key players that need to be taken into consideration when the role of agricultural soils and cultivation practises are evaluated for climate change mitigation. All in all, our study points out how studies on the effects of conservation practices are necessary to fully understand their effects on the environment.

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References

Aira, M., McNamara N.P., Piearce, T.G., and Domínguez, J.: Microbial communities of *Lumbricus terrestris* L. middens: structure, activity and changes through time in relation to earthworm presence, J. Soil. Sediment., 9, 54-61, 2009.

Amador, J.A. and Avizinis, E.J.: Response of nitrous oxide flux to addition of anecic earthworms to an agricultural field, Open J. Soil Sci., 3, 100-106, 2013.

Augustenborg, C.A., Hepp, S., Kammann, C., Hagan, D., Schmidt, O., and Müller, C.: Biochar and earthworm effects on soil nitrous oxide and carbon dioxide emissions, J. Environ. Qual., 41, 1203-1209, 2012.

Bertora, C., van Vliet, P.C.J., Hummelink, E.W.J., and van Groenigen, J.W.: Do earthworms increase N_2O emissions in ploughed grassland? Soil Biol.Biochem., 39, 632-640, 2007.

1 Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven,
2 L., Peres, G., Tondoh, J.E., Cluzeau, D., and Brun J.-J.: A review of earthworm impact on soil
3 function and ecosystem services, Eur. J. Soil Sci., 64, 161-182, 2013.

4 Borken, W., Grundel, S., and Beese, F.: Potential contribution of *Lumbricus terrestris* L. to carbon
5 dioxide, methane and nitrous oxide fluxes from a forest soil, Biol.Fert. Soils, 32, 142-148, 2000.

6 Bradley, R.L., Chroňáková, A., Elhottová, D,. and Šimek, M.: Interactions between land-use history
7 and earthworms control gross rates of soil methane production in an overwintering pasture, Soil
8 Biol. Biochem., 53, 64-71, 2012.

9 Brown, G.G., Barois, I., and Lavelle, P.: Regulation of soil organic matter dynamics and microbial
10 activity in the drilosphere and the role of interactions with other edaphic functional domains, Eur.
11 J. Soil Biol., 36, 177-198, 2000.

12 Butt, K.R.: The effects of temperature on the intensive production of *Lumbricus terrestris* L.
13 (Oligochaeta, Lumbricidae). Pedobiol., 35, 257-264, 1991.

14 Butt, K.R. and Lowe, C.N.: Presence of earthworm species within and beneath *Lumbricus terrestris*
15 L. middens, Eur. J. Soil Biol., 43, S57-S60, 2007.

16 Chan, K.Y.: An overview of some tillage impacts on earthworm population abundance and diversity
17 – implications for functioning in soil, Soil Till. Res., 57, 179-191, 2001.

18 Chen, C., Whalen, J.K., and Guo, X.: Earthworms reduce soil nitrous oxide emissions during drying
19 and rewetting cycles, Soil Biol. Biochem., 68, 117-124, 2014.

20 Davidson, E.A.: Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems, in: Microbial
21 Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes,
22 eds. Rogers, J.E. and Whitman, W.B., American Society for Microbiology, Washington D.C., US,
23 219-235, 1991.

24 Derpsch, R., Friedrich, T., Kassam, A., and Li, H.: Current status of adoption of no-till farming in
25 the world and some of its main benefits, Int. J. Agric. Biol. Eng., 3, 1-25, 2010

26 Devliegher, W. and Verstraete, W.: Microorganims and soil physic-chemical conditions in the
27 drilosphere of *Lumbricus terrestris,* Soil Biol. Biochem., 29, 1721-1729, 1997.

Don, A., Steinberg, B., Schöning, I., Pritsch, K., Joschko, M., Gleixner, G., and Schultze, E.D.: Organic carbon sequestration in earthworm burrows, Soil Biol. Biochem., 40, 1803-1812, 2008.

Drake, H.L. and Horn, M.A.: Earthworms as a transient heaven for terrestrial denitrifying microbes: a review, Eng. Life Sci., 6, 261–265, 2006.

Drake, H.L., Schramm, A., and Horn, M.A.: Earthworm gut microbial biomes: their importance to soil microorganisms, denitrification, and the terrestrial production of the greenhouse gas N_2O, in: Intestinal Microorganisms of Termites and Other Invertebrates, eds. König, H. and Varma, A., Springer, Berlin, 65-87, 2006.

Edwards, C.A. and Lofty, J.R.: The effects of direct drilling and minimal cultivation on earthworm populations, J. Appl. Ecol., 19, 723-734, 1982.

Elliott, P.W., Knight, D., and Anderson, J.M.: Denitrification in earthworm casts and soil from pastures under different fertilizer and drainage regimes, Soil Biol. Biochem., 22, 601-605, 1990.

Evers, A.K., Bambrick, A., Lacombe, S., Dougherty, M.C., Peichl, M., Gordon, A.M., Thevathasan, N.V., Whalen, J., and Bradley. R.L.: Potential greenhouse gas mitigation through temperate tree-based intercropping systems, Open Agric. J., 4, 49-57, 2010.

Fonte, S.J., Kong A.Y.Y., van Kessel, C., Hendrix, P.F., and Six, J.: Influence of earthworm activity on aggregate carbon and nitrogen dynamics differs with agroecosystem management, Soil Biol. Biochem., 39, 1014-1022, 2007.

Giannopoulos, G., Pulleman, M.M., and van Groenigen, J.W.: Interactions between residue placement and earthworm ecological strategy affect aggregate turnover and N_2O dynamics in agricultural soils, Soil Biol. Biochem., 42, 618–625, 2010.

Görres, J.H., Savin, M.C., Nher, D.A., Weicht, T.R., and Amador, J.A.: Grazing in a porous environment: 1. The effect of soil pore structure on C and N mineralization, Plant Soil, 212, 73-85, 1999.

Görres, J.H., Savin, M.C., and Amador, J.A.: Soil micropore structure and carbon mineralization in burrows and casts of an anecic earthworm (*Lumbricus terrestris*), Soil Biol. Biochem., 33, 1881-1887, 2001.

Granli, T. and Bøckman, O.C.: Nitrous oxide from agriculture, Norw. J. Agric. Sci., Supplement 12, 128 p., 1994.

Gregorich, E.G., Rochette, P., St-Georges, P., McKim, U.F., and Chan, C.: Tillage effects on N_2O emission from soils under corn and soybeans in Eastern Canada, Can. J. Soil Sci., 88, 153–161, 2008.

Gumpertz, M.L. and Brownie, C.: Repeated measures in randomized block and split-plot experiments, Can. J. Forest Res., 23, 625-639, 1993.

Gunn, A.: The use of mustard to estimate earthworm populations, Pedobiologia, 36, 65-67, 1992.

Hamilton, W.E. and Sillman, D.Y.: Influence of earthworm middens on the distribution of soil microarthropods, Biol. Fert. Soils, 8, 279-284, 1989.

Hartley, I.P., Heinemeyer, A., and Ineson, P.: Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response, Glob. Chang. Biol., 13, 1761-1770, 2007.

Heinemeyer, A., Di Bene, C., Lloyd, A.R., Tortorella, D., Baxter, R., Huntley, B., Gelsomino, A., and Ineson, P.: Soil respiration: implications of the plant-soil continuum and respiration chamber collar-insertion depth on measurement and modelling of soil CO_2 efflux rates in three ecosystems. Eur. J. Soil Sci., 62, 82-94, 2011.

Henault, C., Devis, X., Page, S., Justes, E., Reau, R., and Germon, J.C.: Nitrous oxide emissions under different soil and land management conditions, Biol. Fert. Soils, 26, 199-207, 1998.

Horn, M.A., Mertel, R., Gehrem, M., Kastner, M., and Drake, H.L.: *In vivo* emission of dinitrogen by earthworms via denitrifying bacteria in the gut, Appl. Environ. Microbiol., 72, 1013-1018, 2006.

Hütsch, B.W.: Methane oxidation in non-flooded soils as affected by crop production, Eur. J. Agron., 14, 237-260, 2001.

Karsten, G.R. and Drake, H.L.: Denitrifying bacteria in the earthworm gastrointestinal tract and *in vivo* emission of nitrous oxide (N_2O) by earthworms, Appl. Environ. Microbiol., 63, 1878-1882, 1997.

Kenward, M.G. and Roger, J.H.: Small sample inference for fixed effects from restricted maximum likelihood, Biometrics 53, 983-997, 1997.

Kerney, M.P. and Cameron, R.A.D.: A Field Guide to Land Snails of Britain and North-West Europe, Collins, London, 1979.

Kladivko, E.: Tillage systems and soil ecology, Soil Till. Res., 61, 61-76, 2001.

Klemedtsson, L., Svensson, B.H., and Rosswall, T.: Relationships between soil moisture content and nitrous oxide production during nitrification and denitrification, Biol. Fert. Soils, 6, 106-111, 1988.

Lal, R.: Residue management, conservation tillage and soil restoration for mitigating greenhouse effect by CO_2-enrichment, Soil Till. Res., 43, 81–107, 1997.

Li, C., Frolking, S., and Butterbach-Bahl, K.: Carbon sequestration in arable soils is likely to increase nitrous oxide emissions, offsetting reductions in climate radiative forcing, Climatic Change, 72, 321–338, 2005.

Loquet, M., Bhatnagar, T., Bouché, M.B., and Rouelle, J.: Essai d'estimation de l'influence écologique des lombriciens sur les microorganisms, Pedobiologia, 17, 400-417, 1977.

Lubbers, I.M., Brussaard, L., Otten, W., and van Groenigen, J.W.: Earthworm-induced N mineralization in fertilized grassland increases both N_2O emissions and crop-N uptake, Eur. J.Soil Sci., 62, 152–161, 2011.

Lubbers, I.M., van Groenigen, K.J., Fonte, S.J., Six, J., Brussaard, L., and van Groenigen, J.W.: Greenhouse-gas emissions from soils increased by earthworms, Nat. Clim. Change, 3, 187-194, 2013a.

Lubbers, I.M., López Gonzalez, E., Hummelink, E.W.J., and van Groenigen, J.W.: Earthworms can increase nitrous oxide emissions from managed grassland: a field study, Agr. Ecosyst. Environ., 174, 40-48, 2013b.

Maraun, M., Alphei, J., Bonkowski, M., Buryn, R., Migge, S., Maren, P., Schaefer, M., and Scheu, S.: Middens of earthworm *Lumbricus terrestris* (Lumbricidae): microhabitats for micro- and mesofauna in forest soil, Pedobiologia, 43, 276-287, 1999.

Matthies, C., Griesshammer, A., Schmittroth, M., and Drake, H.L.: Evidence for involvement of gut-associated denitrifying bacteria in emission of nitrous oxide (N_2O) by earthworms obtained from garden and forest soils, Appl. Environ. Biol., 65, 3599-3604, 1999.

Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T., and Zhang, H.: Anthropogenic and Natural Radiative Forcing, in: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds. Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P.M., Cambridge University Press, Cambridge, 659-740, 2013.

Nieminen, M., Ketoja, E., Mikola, J., Terhivuo, J., Sirén, T., and Nuutinen, V.: Local land use effects and regional environmental limits on earthworm communities in Finnish arable landscapes, Ecol. Appl., 21, 3162–3177, 2011.

Nuutinen, V.: Earthworm community response to tillage and residue management on different soil types in southern Finland, Soil Till. Res., 23, 221-239, 1992.

Nuutinen, V. and Butt, K.R.: Interaction of *Lumbricus terrestris* L. burrows with field subdrains, Pedobiologia, 47, 578-581, 2003.

Nuutinen, V., Butt, K.R., and Jauhiainen, L.: Field margins and management affect settlement and spread of an introduced dew-worm (*Lumbricus terrestris* L.) population, Pedobiologia, 54, S167-S172, 2011.

Palm, C., Blanco-Canqui, H., DeClerck, F., Gatere, L., and Grace, P.: Conservation agriculture and ecosystem sevices: An overview, Agr. Ecosyst. Environ., 187, 87-105, 2014.

Regina, K., Pihlatie, M., Esala, M., and Alakukku, L.: Methane fluxes on boreal arable soils. Agr. Ecosyst. Environ., 119, 346-352, 2007.

Rizhiya, E., Bertora, C., van Vliet, P.C.J., Kuikman, P.J., Faber, J.H., and van Groenigen, J.W.: Earthworm activity as a determinant of N_2O emission from crop residue, Soil Biol. Biochem., 39, 2058-2069, 2007.

Rothwell, A., Chaney, K., and Haydock, P.: The impact of cultivation techniques on earthworm populations, in: Biology of Earthworms, ed. Karaca, A., Springer, Berlin, 159-172, 2011.

Schjønning, P. and Rasmussen, K.J.: Soil strength and soil pore characteristics for direct drilled and ploughed soils, Soil Till. Res., 57, 69–82, 2000.

Schrader, S. and Seibel, C.: Impact of cultivation management in an agroecosystem on hot spot effects of earthworm middens, Eur. J. Soil Biol., 37, 309-313, 2001.

Sharratt, B.S.: Tillage and straw management for modifying physical properties of a subarctic soil, Soil Till. Res., 38, 239–250, 1996.

Sheehy, J., Six, J., Alakukku, L., and Regina, K.: Fluxes of nitrous oxide in tilled and no-tilled boreal arable soils, Agr. Ecosyst. Environ., 164, 190–199, 2013.

Shuster, W.D., Subler, S., and McCoy, E.L.: Foraging by deep-burrowing earthworms degrades surface soil structure of a fluventic Hapludoll in Ohio, Soil Till. Res., 54, 179-189, 2000.

Shuster, W.D., Shipitalo, M.J., Subler, S., Aref, S., and McCoy, E.L.: Earthworm additions affect leachate production and nitrogen losses in typical midwestern agroecosystems, J. Environ. Qual, 32, 2132-2139, 2003.

Sims, R.W. and Gerard, B.M.: Earthworms. Synopses of the British Fauna (New Series) No. 31 (revised), Dorsett Press, Dorchester, 1999.

Six, J. and Paustian, K.: Aggregate-associated soil organic matter as an ecosystem property and a measurement tool, Soil Biol. Biochem., 68, A4-A9, 2014.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., and Sirotenko, O.: Agriculture, in: Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, eds. Metz, B., Davidson, O.R., Bosch, P.R., Dave, R., and Meyer, L.A., Cambridge University Press, Cambridge, 497-540, 2007.

Speratti, A.B. and Whalen, J.K.: Carbon dioxide and nitrous oxide fluxes from soil as influenced by anecic and endogeic earthworms, Appl. Soil Ecol., 38, 27-33, 2008.

Stockmann, U., Adams, M.A., Crawford J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., de Courcelles, V., Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., and Zimmermann, M.: The knowns, known unknowns and unknowns of sequestration of soil organic carbon, Agr. Ecosyst. Environ., 164, 80-99, 2013.

Subler, S. and Kirsch, A.S.: Spring dynamics of soil carbon, nitrogen, and microbial activity in earthworm middens in a no-till cornfield, Biol. Fert. Soils, 26, 243-249, 1998.

Šustr, V. and Šimek, M.: Methane release from millipedes and other soil invertebrates in Central Europe, Soil Biol. Biochem., 41, 1684-1688, 2009.

Svensson, B.H., Bostrom, U., and Klemedtsson, L.: Potential for higher rates of denitrification in earthworm casts than in the surrounding soil, Biol. Fert. Soils, 2, 147–149, 1986.

Tebrügge, F. and Düring R.-A.: Reducing tillage intensity – a review of results from a long-term study in Germany, Soil Till. Res., 53, 15–28, 1999.

Theenhaus, A. and Scheu, S.: The influence of slug (*Arion rufus*) mucus and cast material addition on microbial biomass, respiration, and nutrient cycling in beech leaf litter, Biol. Fertil. Soils, 23, 80-85, 1996.

Tiunov, A.V. and Scheu, S.: Microbial respiration, biomass, biovolume and nutrient status in burrow walls of *Lumbricus terrestris* L. (Lumbricidae), Soil Biol. Biochem., 31, 2039-2048, 1999.

van Groenigen, J.W., Lubbers, I.M., Vos, H.M.J., Brown, G.G., De Deyn, G.B., and van Groenigen, K.J.: Earthworms increase plant production: a meta-analysis, Scientific Reports, 4, 6365, 2014.

Whalen, J.K and Fox, C.A.: Diversity of lumbricid earthworms in temperate agroecosysstem, in: Biodiversity in Agricultural Production Systems, eds. Benckiser, G. and Schnell, S., Taylor & Francis, Boca Raton, 249-261, 2007.

Wilcox, C.S., Domínguez, J., Parmelee, R.W., and McCartney, D.A.: Soil carbon and nitrogen dynamics in *Lumbricus terrestris* L. middens in four arable, a pasture, and a forest ecosystems, Biol. Fert. Soils, 36, 26-34, 2002.

Wu, D., Liu, M., Song, X., Jiao, J., Li, H., and Hu, F.: Earthworm ecosystem service and dis-service in a N-enriched ecosystem: Increase of plant production leads to no effects on yield-scaled N_2O emissions, Soil Biol. Biochem., 82, 1-8, 2015.

Wüst, P.K., Horn, M.A., and Drake, H.L.: *In situ* hydrogen and nitrous oxide as indicators of concomitant fermentation and denitrification in the alimentary canal of the earthworm *Lumbricus terrestris,*. Appl. Environ. Microbiol., 75, 1852-1859, 2009.

Zhang, W., Hendrix, P.F., Dame, L.E., Burke, R.A., Wu, J., Neher, D.A., Li, J., Shao, Y., and Fu, S.: Earthworms facilitate carbon sequestration through unequal amplification of carbon stabilization compared with mineralization, Nat. Comm., 4, 2576, 2013.

Table 1. Fixed effect (treatment and site) *P*-values of general linear mixed models with repeated measurements (date) for N_2O, CO_2 and CH_4 emissions in the field and laboratory measurements. Treatment is 'midden area vs. non-midden area' in the field and '*L. terrestris* vs. control' in the laboratory mesocosms.

	Model term	N_2O	CO_2	CH_4
Field	Site			0.008
	Treatment	<0.001	<0.001	0.043
	Treatment × Site			0.072
	Date	0.004	<0.001	0.029
	Site × Date		<0.001	
	Treatment × Date	0.289	0.588	<0.001
	Treatment × Site × Date			0.007
Laboratory	Treatment	<0.0001	<0.0001	0.482
	Date	<0.0001	<0.0001	0.144
	Treatment × Date	0.159	0.401	0.039

Table 2. The mean estimates (SE) of statistical models for cumulative N_2O, CO_2 and CH_4 fluxes in the field (duration 2 weeks) and laboratory (15 weeks) measurements.

	N_2O	CO_2	CH_4
	μg N chamber area^{-1}	mg chamber area^{-1}	μg chamber area^{-1}
Field:			
Midden area	74.2 (5.1)	591.4 (28.4)	-2.6 (1.1)
Non-midden area	47.6 (5.1)	394.4 (28.4)	-4.8 (1.1)
Laboratory:			
L. terrestris	111.3 (7.1)	3224 (157)	-230.7 (9.2)
Control	90.3 (6.2)	2729 (152)	-224.7 (8.1)

Table 3. Fixed effect (site and treatment) *P*-values of general linear mixed models with repeated measurements (midden and residue removal) for N_2O, CO_2 and CH_4 emissions in the field and laboratory measurements. Treatment is 'midden area vs. non-midden area' in the field and '*L. terrestris* vs. control' in the laboratory mesocosms.

	Model term	N_2O	CO_2	CH_4
Field	Site			0.007
	Treatment	0.012	0.009	0.015
	Treatment × Site			0.080
	Removal	0.401	0.980	0.139
	Site × Removal			0.034
	Treatment × Removal	0.845	0.338	0.176
	Treatment × Site × Removal			0.894
Laboratory	Treatment	0.083	0.002	0.886
	Removal	0.004	0.008	0.440
	Treatment × Removal	0.449	0.054	0.317

Table 4. Characteristics of *L. terrestris* midden (n=10) and adjacent non-midden areas (n=10) at the end of the field measurements (model based mean estimates with 95% confidence intervals presented for all other variables except for the slug *Arion fasciatus*, which has medians with a minimum and maximum). *F*- and *P*-statistics show the statistical significance of the difference between the midden and non-midden areas (for slugs the values are from non-parametric Wilcoxon signed rank test).

	Midden area	Non-midden area	df	*F*	*P*
Earthworm number [a]	3.6 (2.6-4.6)	1.6 (0.6-2.6)	1, 8	8.51	0.019
Earthworm mass (g f.w.) [a]	2.0 (1.4-2.7)	0.8 (0.1-1.5)	1, 16	7.81	0.013
Slug number [a]	3.0 (0, 6)	0 (0, 1)		22.5	0.004
Soil moisture (% of f.w.) [b]	26.5 (25.8-27.2)	25.4 (24.8-26.1)	1, 8	7.66	0.024
Mineral N (mg kg^{-1} soil d.w.) [b]	9.2 (7.9-10.5)	7.1 (5.7-8.4)	1,8	8.24	0.021
Potential denitrification ($\mu g\ N_2O\text{-}N\ g^{-1}$ soil d.w. h^{-1}) [b]	1.2 (1.1-1.4)	1.0 (0.9-1.2)	1,8	4.16	0.076

[a] Sample covers the chamber base area (diameter 15 cm)

[b] Soil core (depth 5 cm, diameter 5 cm); in the midden area taken around the *L. terrestris* burrow entrance.

Table 5. Characteristics of *L. terrestris* (n=13) and control mesocosms (n=15) at the end of the laboratory experiment (model based mean estimates and 95% confidence intervals presented for all variables). *F*- and *P*-statistics show the statistical significance of the difference between the *L. terrestris* and control mesocosms.

	L. terrestris	Control	df	*F*	*P*
Mineral N (mg kg^{-1} soil d.w.) [a]	21.9 (20.9-23.0)	21.0 (20.0-21.9)	1, 12.3	8.71	0.012
Soil moisture (% of f.w.) [b]	20.7 (20.6-20.8)	20.4 (20.3-20.5)	1, 14.1	13.46	0.003
Mineral N (mg kg^{-1} soil d.w.) [b]	23.1 (21.0-25.4)	19.3 (17.6-21.2)	1, 24	7.74	0.010
Potential denitrification ($\mu g\ N_2O\text{-}N\ g^{-1}$ soil d.w. h^{-1}) [b]	0.30 (0.27-0.32)	0.25 (0.23-0.27)	1, 26	10.55	0.003

[a] Sample represents the entire soil column (excluding the soil core)

[b] soil core (depth 5 cm, diameter 5 cm); in the *L. terrestris* mesocosm taken around the burrow entrance

Figure captions

Figure 1. The mean (±SE) estimates of statistical models for (a) N_2O, (b) CO_2 and (c, d) CH_4 (separately for field sites A and B) emissions in *L. terrestris* midden (●) and non-midden (o) areas and the effect of the removal of middens and surface residues on the emissions. For CH_4, the differences between the midden and non-midden areas at $p<0.05$ are marked with * (for effects on N_2O and CO_2, see Table 1).

Figure 2. The mean estimates (±SE) of statistical models for (a) N_2O, (b) CO_2 and (c) CH_4 emissions in *L. terrestris* (●) and control (o) mesocosms and the effect of the removal of middens and surface residues on the emissions. For CH_4, the differences between treatments at $p<0.1$ are marked with * (for effects on N_2O and CO_2, see Table 1).

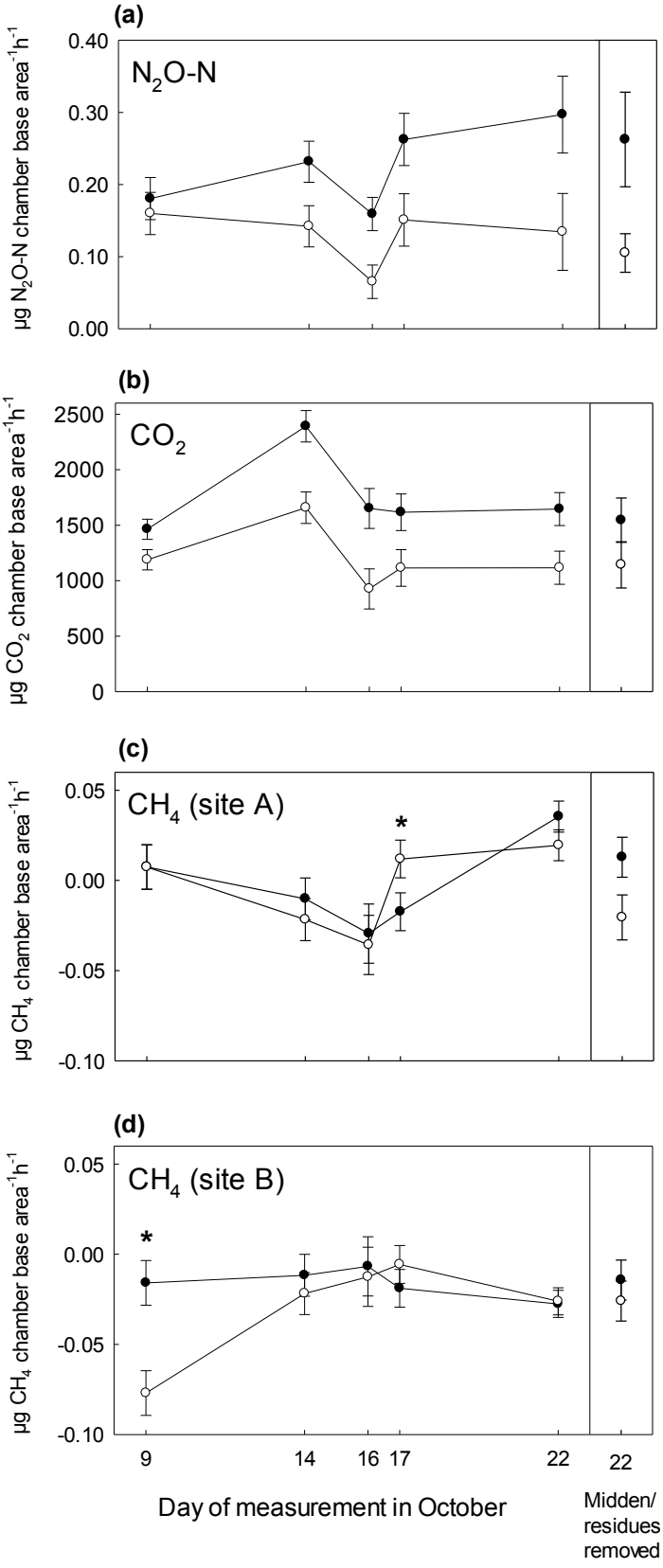
(a)
N2O-N
µg N2O-N chamber base area-1h-1
0.40
0.30
0.20
0.10
0.00
(b)
CO2
µg CO2 chamber base area-1h-1
2500
2000
1500
1000
500
0
(c)
CH4 (site A)
µg CH4 chamber base area-1h-1
0.05
0.00
-0.05
-0.10
*
(d)
CH4 (site B)
µg CH4 chamber base area-1h-1
0.05
0.00
-0.05
-0.10
*
9
14
16
17
22
22
Day of measurement in October
Midden/
residues
removed

Fig 1.

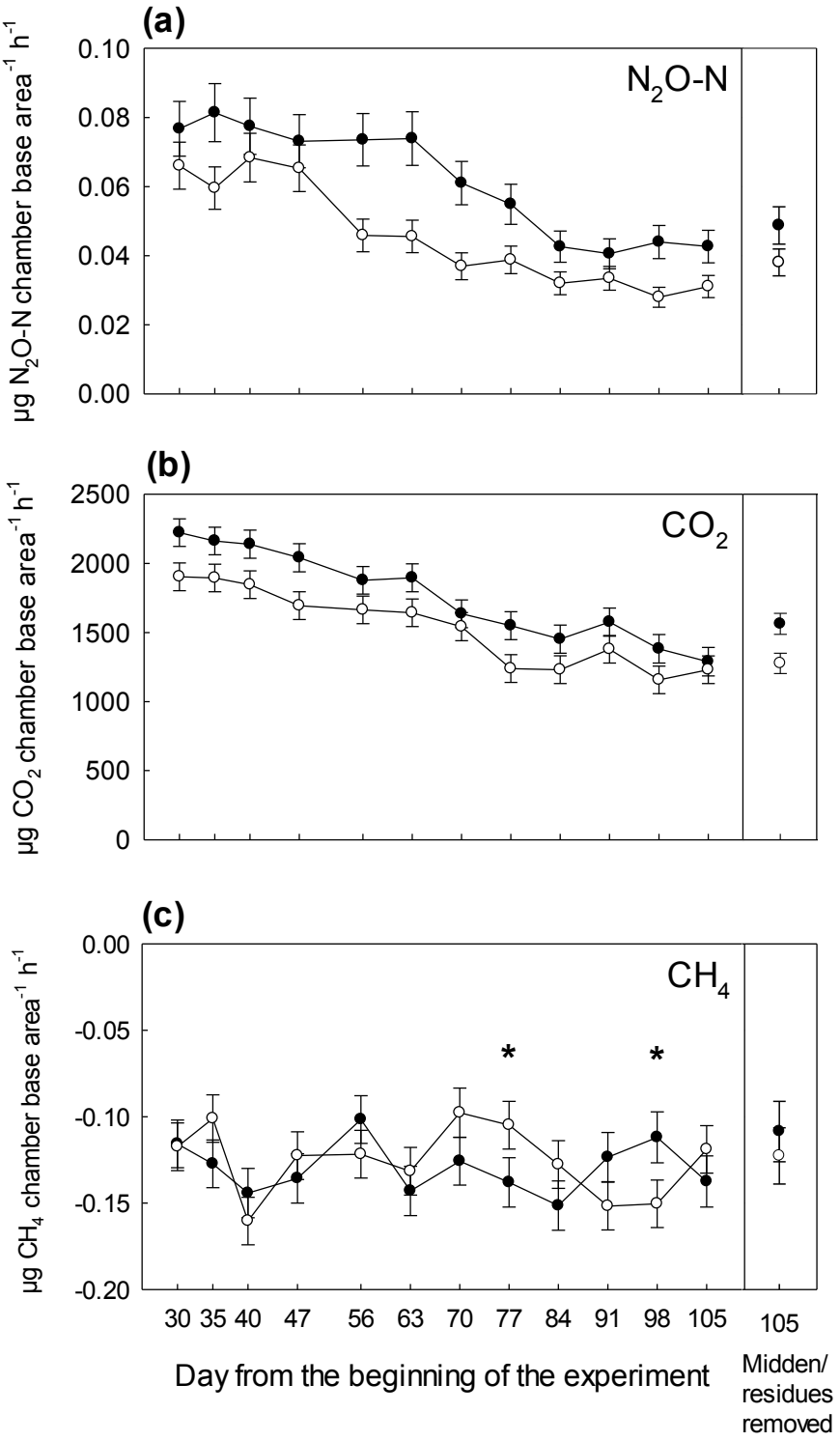
(a)
N_2O-N
µg N_2O-N chamber base area^{-1} h^{-1}
0.10
0.08
0.06
0.04
0.02
0.00
(b)
CO_2
µg CO_2 chamber base area^{-1} h^{-1}
2500
2000
1500
1000
500
0
(c)
CH_4
µg CH_4 chamber base area^{-1} h^{-1}
0.00
-0.05
-0.10
-0.15
-0.20
*
*
30 35 40 47 56 63 70 77 84 91 98 105
105
Day from the beginning of the experiment
Midden/
residues
removed

Fig. 2