

## Carbon dioxide and methane exchange of a perennial grassland on a boreal mineral soil

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Cultivation of perennial crops can be an option to sequester carbon in agricultural soils. To determine the carbon budget of a perennial cropping system under the boreal climate, we studied carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) exchange of timothy and meadow fescue mixture (TIM) on a boreal mineral soil. Based on the mean annual net ecosystem CO<sub>2</sub> exchange (NEE), TIM was a sink for both CO<sub>2</sub> (–1000 g CO<sub>2</sub> m<sup>–2</sup>) and CH<sub>4</sub> (–140 mg CH<sub>4</sub> m<sup>–2</sup>). In comparison, soil without vegetation (BARE) was a source of CO<sub>2</sub> (1300 g CO<sub>2</sub> m<sup>–2</sup>). Based on the literature review, the net CO<sub>2</sub> uptake of TIM was similar to the perennial cropping systems in northern Finland but higher than that of the annual cropping systems in this region. Our multi-year study shows that the perennial cultivation system based on TIM is an environmentally sustainable land-use option to mitigate agricultural CO<sub>2</sub> emissions in regions with short growing seasons.

### Introduction

An increase in the concentrations of greenhouse gases (GHGs), including carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), in the atmosphere is causing global climate change (Ciais *et al.* 2013). The atmospheric content of CO<sub>2</sub> was 400 ppm and that of CH<sub>4</sub> was 1845 ppb in 2015 (WMO 2016). Deforestation and our excessive dependence on fossil fuels are among the key reasons for the increasing CO<sub>2</sub> levels

in the atmosphere (Andres *et al.* 1999, Bonan 2008). The increase in the concentration of CH<sub>4</sub> in the atmosphere is associated with human activities, such as fossil fuel industry, agriculture and landfills (Nisbet *et al.* 2014, Schwietzke *et al.* 2017). The Paris climate agreement adopted in December 2015 aims at holding global temperature rise to below 2°C of the preindustrial level and pursuing efforts to limit it further to 1.5°C. Therefore, attempts to reach these targets are in progress.

The accurate quantification of agricultural GHG emissions and implementation of mitigation activities pose a challenge. From a mitigation viewpoint, perennial crops are preferred over annual ones, as perennial systems are considered to have environmental benefits (e.g., Saarijärvi *et al.* 2004, Dohleman and Long 2009, DuPont *et al.* 2010). The energy inputs related to the machinery are lower for perennial crops because the soil is neither tilled annually nor the crop established annually. Thus, perennial crops have a great potential to capture and store carbon as they grow over the entire growing season (Dohleman and Long 2009) and have high root biomass (DuPont *et al.* 2010). The root-derived carbon compounds decompose at a slower rate than those from the above-ground biomass (Kätterer *et al.* 2011). Furthermore, the erosion risk and nutrient run-off are lower compared to annual crops (Saarijärvi *et al.* 2004).

The most important perennial grass species in the boreal region are timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) (Niskanen and Niemeläinen 2010), as they are well adapted to the boreal environment. Timothy is native to northern Europe (Casler and Kallenbach 2007), whereas meadow fescue is native to temperate and northern areas. Timothy and meadow fescue are often cultivated as a mixture in Finland (Niskanen and Niemeläinen 2010). They grow to a 0.5 m to 1.0 m in height in a boreal climate and they can be harvested two to three times per season (Virkajärvi *et al.* 2015). Their annually harvestable yield varies from 6.3 t to 8.3 t dry matter (DM) ha<sup>-1</sup> in Finland depending on the fertilization rates, the number of harvests per season and the age of the grassland (Nissinen and Hakkola 1994). The grass mixture is used not only as fodder for cattle, but also as hay and substrate for biogas reactors (Lehtomäki *et al.* 2008). The rotation time of a managed grassland in Finland is short — on average four to five years (Virkajärvi *et al.* 2015). There is a decrease in the yield after the third season mainly due to damages in the winter and an increase in the low productive weeds (Nissinen and Hakkola 1994).

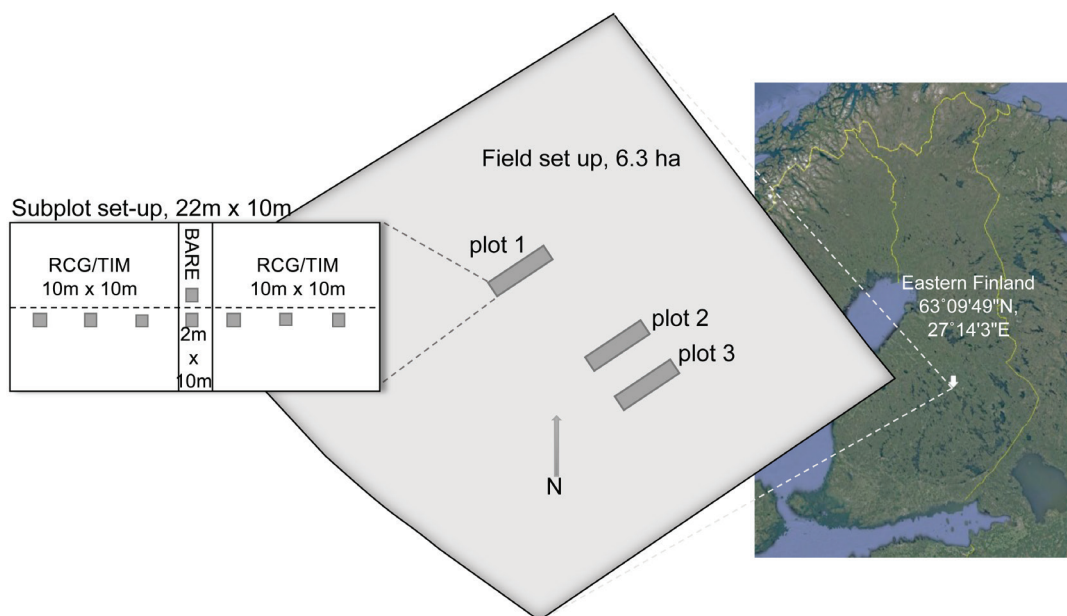
The role of agricultural soils in climate change mitigation is contradictory. While bioenergy is widely used as a renewable energy

source, bioenergy crop production can lead to a reduction of the soil carbon stock and an increase in N<sub>2</sub>O emissions (Crutzen *et al.* 2008, Creutzig *et al.* 2015). However, agricultural soil can also be a sink of atmospheric CO<sub>2</sub>. The initiative, “4 per 1000” ([www.4p1000.org](http://www.4p1000.org)) aims to increase the carbon storage in agricultural soils and thus reducing CO<sub>2</sub> emissions and increasing food security. For agricultural soils, this is achieved by modifying agricultural practices, such as omitting to keep soil without vegetation, increasing the use of perennial crops, increasing the amount of crop residues left in the soil and using organic fertilizers. To estimate the impact of adopted management practices on GHG emissions in a given cropping system, studies on GHG exchange are needed. Although the mixture of timothy and meadow fescue (TIM) cropping is economically important as fodder for cattle (dairy milk and beef production) and substrate for biogas reactors, its atmospheric impact is poorly known. Therefore, we initiated a multi-year (2009–2011) study on greenhouse gas exchange of TIM cultivated on a boreal mineral soil. Here, we aim to quantify the annual CO<sub>2</sub> and CH<sub>4</sub> budget of this perennial cropping system and investigate factors controlling the exchange of these greenhouse gases.

## Material and methods

### Study site

The study site, located in Maaninka (63°09'49"N, 27°14'3"E, 89 m asl) in eastern Finland, is described in detail in Lind *et al.* (2016). In brief, the long-term annual air temperature is 3.2°C with an annual precipitation of 612 mm (30 years, reference period 1981–2010; Pirinen *et al.* 2012). The experimental site is a 6.3 ha agricultural field. The soil is classified as a Haplic Cambisol/Regosol (Hypereutric, Siltic) (IUSS Working Group WRB 2007), the topsoil being silt loam (clay 25% ± 6%, silt 53% ± 9% and sand 22% ± 8%) according to the U.S. Department of Agriculture (USDA) textural classification system with soil organic matter content of 5.2% and organic matter content of 3.0%.



**Fig. 1.** General location of the study site and the set-up at field and subplot levels. Three plots (grey rectangle) were placed in the middle section of the field. The plots were further divided into subplots. On each subplot, one end (10 m × 10 m area) was cultivated with a mixture of timothy and meadow fescue (TIM) and another with reed canary grass (RCG). Order of the TIM and RCG varied. Between of the two vegetated parts, a 2 m × 2 m area was kept without vegetation (BARE). Grey squares in the subplots mark the location of the collars used for flux measurements. Data from RCG are not shown. Map data © 2018 Google.

The experimental design consisted of three plots (10 m × 22 m). They were established within the main field in June 2009. Each of the plots was divided into three subplots (Fig. 1) that were cultivated with either reed canary grass (Lind *et al.* 2016) or a mixture of timothy (*Phleum pratense*, cv. "Tuure") and meadow fescue (*Festuca pratensis*, cv. "Antti"; mixture referred hereafter as TIM). The third treatment was a bare soil without any growing vegetation (referred hereafter as BARE). The order of the treatments was randomized but BARE subplots were located between the crop treatments to prevent the spread of the plants and their roots from one treatment to another.

Normal agricultural practices were followed. Timothy (seed rate at 12 kg ha<sup>-1</sup>) and meadow fescue (seed rate at 10 kg ha<sup>-1</sup>) were sown together with barley (*Hordeum vulgare*, cv. "Voitto"; seed rate at 120 kg ha<sup>-1</sup>) as a cover crop in the establishment year (2009). Barley was removed from TIM in the first harvest. Mineral fertilizers were applied while seeding

in 2009 and as surface applications at subsequent times (Table 1). Herbicide (a mixture of MPCA 200 g l<sup>-1</sup>, clopyralid 20 g l<sup>-1</sup> and fluroxypyr 40 g l<sup>-1</sup>, 2 l with 200 l of water ha<sup>-1</sup>) was applied by the end of July 2009 to control the weeds on TIM. TIM was harvested using a plot harvester (Haldrup 1500 plot harvester, Løgtør, Denmark) once in 2009 and twice in 2010 and 2011 (Table 1). Any plants growing on BARE were hand-picked once a week during the growing season to keep the plots vegetation-free. No herbicides nor fertilizers were applied on BARE.

## Gas exchange

To cover the temporal variations in fluxes, we applied season-specific manual flux measurement methods. During snow-covered seasons, a snow-gradient method (Sommerfeld *et al.* 1993) was used for the CO<sub>2</sub> and CH<sub>4</sub> exchange. During snow-free seasons, CO<sub>2</sub> exchange and respira-

tion components were measured using a static chamber method employing transparent and opaque chambers (Alm *et al.* 1997) and  $\text{CH}_4$  exchange with an opaque static chamber method with permanent collars (Nykänen *et al.* 1995). During April to June in 2009, when the soil was bare and plots were not set up yet,  $\text{CO}_2$  and  $\text{CH}_4$  fluxes were measured with an opaque static chamber method without collars (Maljanen *et al.* 2006). An opaque cylinder chamber was pressed 2–5 cm deep in the soil for the gas flux measurement and removed after the measurement. This was done to characterize the background GHG emissions from the study area.

After the experimental plots were established, aluminum collars (60 cm  $\times$  60 cm  $\times$  15 cm) with water grooves were installed on the plots for measurements of ecosystem respiration (TER), net ecosystem  $\text{CO}_2$  exchange (NEE) and  $\text{CH}_4$  fluxes. Three collars were installed on each TIM plot and two on each BARE plot. Collars made of PVC ( $\varnothing$ 10.4 cm) were installed close to the aluminum collars for measurements of soil respiration (SR) and belowground respiration (BR) on TIM. Collars for SR were installed down to 15 cm depth and collars for BR to 3 cm depth. In SR, we assumed that the measurements represent the  $\text{CO}_2$  emissions originating from the soil as the collar itself prevented the growth of the roots of adjacent plants inside the collar. In BR, we assumed that the  $\text{CO}_2$  emissions originated from the soil and roots of the adjacent plants as the low collar depth did not interfere with the root growth. In both systems, living plants were removed by hand-picking.

The gas exchange of  $\text{CO}_2$  and  $\text{CH}_4$  throughout the snowpack was determined using gas samples drawn from the snowpack at 10 cm

intervals until 2 cm above the soil surface with a metal probe (length 0.5 m or 1.2 m,  $\varnothing$ 2 mm) connected to a 60 ml polypropylene syringe. Ambient samples were collected 2 cm above the snowpack. The gas samples (30 ml) were injected from the syringe into pre-evacuated 12 ml glass vials (Labco Exetainer®) and analysed within a month using a gas chromatograph (GC, Agilent 6890N, Agilent Technologies Deutschland, Germany) equipped with flame ionization detector (FID) for  $\text{CH}_4$  and electron capture detector (ECD) for  $\text{CO}_2$  with known standards. The calculation of flux rates was based on the concentration gradients and diffusion rates of  $\text{CO}_2$  and  $\text{CH}_4$  in the snow. A diffusion coefficient of  $0.22 \text{ cm}^2 \text{ s}^{-1}$  for  $\text{CO}_2$  and  $0.14 \text{ cm}^2 \text{ s}^{-1}$  for  $\text{CH}_4$  was used (Sommerfeld *et al.* 1993). For diffusion calculations, snow samples were collected from three locations per treatment using a PVC tube ( $\varnothing$ 9 cm). The snow samples were weighted and the snow porosity was calculated using the density of pure ice ( $0.92 \text{ g cm}^{-3}$ ). After visual inspection of the data, high fluxes were accepted when the gas concentration change with depth was considered linear ( $R^2 > 0.7$ ). To omit bias in the mean flux calculations, low fluxes ( $\pm 30 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  and  $\pm 20 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ ) were accepted regardless their  $R^2$  values. Approximately 20% of the  $\text{CO}_2$  and 5% of the  $\text{CH}_4$  data were rejected.

During the snow-free season, daytime methane ( $\text{CH}_4$ ) exchange was measured using opaque chambers with extra collars to omit damage of the tall vegetation during the measurements. Four gas samples were taken during the chamber closure time, varying from 28–60 minutes; and being longer with greater volumes when extra collars were applied (see

**Table 1.** Rates ( $\text{kg ha}^{-1}$ ) of nitrogen (N), phosphorus (P) and potassium (K) fertilization together with the harvesting dates and the yields as dry weight ( $\text{kg DW ha}^{-1}$ ) of a mixture of timothy and meadow fescue (TIM). TIM was harvested and fertilized once in 2009 and twice in 2010 and 2011 (yields of individual harvests in brackets).

| Year | Fertilization date | N ( $\text{kg ha}^{-1}$ ) | P ( $\text{kg ha}^{-1}$ ) | K ( $\text{kg ha}^{-1}$ ) | Harvesting date  | Yield ( $\text{kg DW ha}^{-1}$ ) |
|------|--------------------|---------------------------|---------------------------|---------------------------|------------------|----------------------------------|
| 2009 | 9 Jun.             | 60                        | 30                        | 45                        | 21 Aug.          | 6400                             |
| 2010 | 21 May; 30 Jun.    | 100; 100                  | 15; 0                     | 25; 35                    | 22 Jun.; 23 Aug. | 13 000 (7200; 5700)              |
| 2011 | 27 May; 7 Jul.     | 100; 100                  | 15; 0                     | 25; 35                    | 22 Jun.; 5 Sept. | 14 000 (7400; 6400)              |

above). The gas was sampled with a syringe, transferred and stored in a pre-evacuated vial for subsequent analysis (see above) using a GC. Fluxes were calculated from the linear change during the closure time. After visual inspection of the data, high fluxes with  $R^2 > 0.8$  were accepted. To omit bias in the mean flux calculations, low fluxes (between  $\pm 40 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$  and  $\pm 170 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ , depending on closure time and volume) were accepted regardless of their  $R^2$  values. About 7% of the measured fluxes were rejected. Methane released to the atmosphere is defined as positive and uptake from the atmosphere as negative.

NEE was measured during the snow-free season with a transparent polycarbonate chamber ( $60 \text{ cm} \times 60 \text{ cm} \times 30 \text{ cm}$ ), equipped with a fan and ice water cooling system to keep the chamber temperature close to the prevailing air temperature. An infrared gas analyzer (IRGA; model: LI-840, LiCor) was used for the analysis of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  concentrations in the chamber during the 2 minute closure time (for details, see Marushchak *et al.* 2013). Air temperature ( $T_A$ ; model: 107, Campbell Scientific Inc.) and photosynthetically active radiation (PAR; model: SKP215, Skye Instruments) inside the chamber were recorded. Additional measurements of NEE under reduced light conditions were done in 2010 and 2011 by shading the chamber with a net. Measurement of TER was done using an opaque cover on the transparent chamber after the measurement of NEE. Extra collars were used to omit the disturbance during the measurements when plants grew taller.

Daytime SR and BR were measured with an opaque PVC chamber ( $\varnothing 11.2 \text{ cm}$ , volume  $1.1 \text{ dm}^3$ ) using IRGA during the 1.5 minute closure time. Fluxes were calculated from the change in the  $\text{CO}_2$  concentration in the chamber headspace with a MATLAB (R2010a, MathWorks) program using the exponential non-linear model (Kutzbach *et al.* 2007). After initial visual inspection, the residual standard deviation of the regression  $< 2 \text{ ppm}$  was used as a data quality control criterion. About 9% of the data were rejected. Gross primary productivity (GPP) was calculated based on NEE and TER ( $\text{GPP} = \text{NEE} - \text{TER}$ ). Carbon dioxide released to the atmosphere is

defined as positive and uptake from the atmosphere as negative.

## Supporting measurements

Climatic variables were recorded by a weather station in the field. The supporting meteorological measurements included  $T_A$  (model: HMP45C, Vaisala Inc), PAR (model: SKP215, Skye Instruments Ltd.), amount of rainfall measured at 1 m height (model: 52203, R.M. Young Company) and air pressure (model: CS106 Vaisala PTB110 Barometer). Data were collected using a datalogger (model: CR 3000, Campbell Scientific Inc.). Supporting data collection began on 14 August 2009. Short gaps in the data were filled using linear interpolation and longer gaps with data from Maaninka weather station operated by the Finnish Meteorological Institute (FMI) about 6 km south-east from the site.

Leaf area index (LAI) and plant height were measured weekly. LAI was measured using a plant canopy analyzer (model: LAI-2000, LiCor) with a  $180^\circ$  view cap from plots in 2010 and 2011. To determine the daily plant height, data were fitted using a quadratic polynomial function.

Soil temperature ( $T_s$ , model: 109, Campbell Scientific Inc., UK) and volumetric water content (VWC, model: CS616, Campbell Scientific Inc., UK) in the soil profile were continuously recorded on TIM. Data were collected using a datalogger (CR200, Campbell Scientific Inc.). On BARE, soil temperature was recorded using iButtons® (model: DS1921G, Maxim Integrated Products, Inc. USA). Data were collected from July 2009 onwards. Concurrently with the flux measurements, soil temperatures at 0 cm, 2 cm, 5 cm, 10 cm, 15 cm and 20 cm depths were measured with a temperature probe and the VWC from 0–7 cm depth with a moisture meter (HH2 equipped with ThetaProbe ML2x: Delta-T Devices Ltd.) adjacent to the flux measurement point.

Soil  $\text{CO}_2$  and  $\text{CH}_4$  concentrations were followed from September 2009 onwards. Concentrations at 5 cm, 20 cm and 30 cm depths in the soil were determined using PVC soil gas col-



lectors (Kammann *et al.* 2001, Ø1.4 cm, length 50 cm, Ø of holes were 3 mm). Gas samples were collected approximately twice a month. Gas samples were stored in vials and analyzed subsequently with a GC.

During winter, soil frost (methylene blue method, Gandahl 1957) and snow depth were measured manually on TIM at a weekly interval. On BARE, only snow depth was measured.

## Flux partitioning methods

Ecosystem respiration was partitioned into root respiration (RR), soil respiration (SR) and aboveground respiration (AGR). Root respiration was calculated from measured values of SR and BR (RR = BR – SR). The aboveground respiration (AGR) was calculated from TER as follows: AGR = TER – RR – SR.

The relationships between C fluxes and environmental variables were analyzed by correlation analyses. The normal distribution of the data was checked using the Kolmogorov-Smirnov test (e.g., Yap and Sim 2011). As most of the data were not normally distributed, correlation analysis was carried out using the Spearman's rank correlation (Glasser and Winter 1961). The correlations during the summer period (June–September 2009, May–September 2010 and May–September 2011) between C fluxes and environmental variables (PAR,  $T_A$ ,  $T_S$  (0 cm, 2 cm, 5 cm, 10 cm, 15 cm and 20 cm depths), VWC, CO<sub>2</sub>/CH<sub>4</sub> concentration (5 cm, 20 cm and 30 cm depths) in soil) and descriptors of plant productivity (plant height and LAI) were tested. During wintertime (November 2009–April 2010 and November 2010–April 2011), tested variables were  $T_A$ , snow temperature 2 cm above the ground,  $T_S$  (5 cm, 10 cm and 20 cm depths), snow depth, frost depth, snow porosity and CO<sub>2</sub>/CH<sub>4</sub> concentrations (5 cm, 20 cm and 30 cm depths) in soil. The correlation was considered meaningful when the coefficient was higher than 0.6 and the correlation was statistically significant ( $p < 0.05$ ). All analyses were conducted using IBM SPSS Statistics ver. 21.

The non-linear relationship between TER and  $T_A$  and between GPP and PAR during the

summer period were analyzed. TER data were binned with  $T_A$  and the bin-averaged values of TER were plotted against  $T_A$  and the data were fitted with an exponential regression of the form (e.g., Shurpali *et al.* 2009):

$$\text{TER} = R_{10} \times Q_{10}^{\left(\frac{T_A}{T_{10}}\right)}, \quad \text{Eq. 1}$$

where  $T_A$  is the measured air temperature,  $T_{10}$  is 10°C and the fitted parameters are  $R_{10}$  (base respiration; mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) and  $Q_{10}$  (temperature sensitivity). GPP data were binned with PAR and the bin-averaged values of GPP were plotted against PAR and the data were fitted with a rectangular hyperbolic model of the form (e.g., Thornley and Johnson 1990):

$$\text{GPP} = \frac{\text{GP}_{\max} \times \text{PAR} \times \alpha}{\text{GP}_{\max} + \text{PAR} \times \alpha}, \quad \text{Eq. 2}$$

where  $\text{GP}_{\max}$  (μmol m<sup>-2</sup> s<sup>-1</sup>, the theoretical maximum rate of photosynthesis at infinite PAR) and  $\alpha$  (apparent quantum yield) are model parameters.

Annual values were constructed from the measured data. Non-linear regression models were used to model GPP and TER during summer periods (June–September 2009 and May–September 2010 and 2011) separately for each of the plots. The respiration model was based on the air temperature (Eq. 1). For GPP, two models were created. The first model was based only on PAR (Eq. 2) and the second considered also the plant height variation as shown in the following form:

$$\text{GPP} = \frac{\text{GP}_{\max} \times \text{PAR} \times \alpha}{\text{GP}_{\max} + \text{PAR} \times \alpha} + (c + d \times \text{height}), \quad \text{Eq. 3}$$

where  $c$  and  $d$  are scaling parameters and height is plant height. The range of the fit results is shown for TER and GPP in the appendix (Appendix Table A1 and Appendix Table A2). The two models differed in their predictive power and the model with the best predictive power was used for further analyses. During the snow-covered periods, linear interpolation was used to determine TER. The linear interpolation was also used for CH<sub>4</sub> exchange. Annual values were calculated by summing up the flux

values. Finally,  $\text{CH}_4$  fluxes were converted to  $\text{CO}_2$ -equivalents using a factor of 28 for the 100-year time horizon (Ciais *et al.* 2013).

## Results

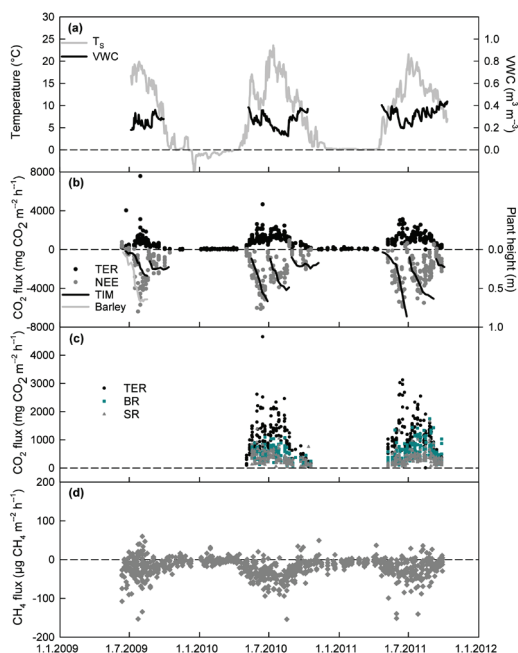
### Weather conditions

The mean annual air temperature at the study site was  $3.4^\circ\text{C}$ ,  $2.0^\circ\text{C}$  and  $4.4^\circ\text{C}$  and the annual precipitation was 420 mm, 520 mm and 670 mm in 2009, 2010 and 2011, respectively (Appendix Table A3). July was always the warmest month. December was the coldest month in 2009 and January in 2010 and 2011 (Appendix Table A3). The growing season is defined to start when the mean daily air temperature exceeds  $5^\circ\text{C}$  with no snow, and it ends when the mean daily air temperature is below  $5^\circ\text{C}$  for five consecutive days. The length of the growing season was 152, 156 and 182 days in 2009, 2010 and 2011, respectively.

### Carbon dioxide dynamics

A clear seasonal pattern of net ecosystem  $\text{CO}_2$  exchange (NEE) and ecosystem respiration (TER) was observed on a mixture of timothy and meadow fescue (TIM). NEE was low at the beginning and the end of the growing seasons. Crop harvest caused, as expected, an abrupt drop in the  $\text{CO}_2$  uptake. NEE peaked at about  $-6000 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  in each year. The range of the measured TER rates was higher during the May to September periods than during the snow-covered season (Fig. 2b). During the snow-covered seasons, TER was, in general, below  $100 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ . The TER peak varied between  $2000 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  and  $3000 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  during May to September.

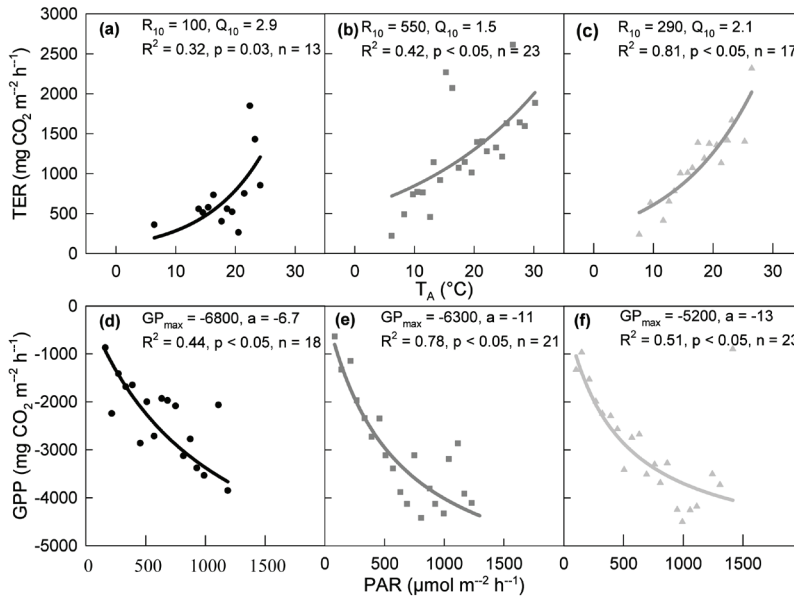
Based on the measured soil respiration (SR) and belowground respiration (BR) data on TIM, both SR and BR rates had peaks of about  $1000 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  in 2010 and 2011. Using these measured data, TER was partitioned into SR, root respiration (RR) and aboveground respiration (AGR). The seasonal mean of the respiration components increased from RR to SR with



**Fig. 2.** Seasonal variation of the measured variables on a mixture of timothy and meadow fescue (TIM). (a) Daily mean soil temperature ( $T_s$ , grey line) from July 2009 to October 2011 and volumetric water content (VWC, black line) for growing season in 2009, 2010 and 2011 at 5cm depth and weekly plant height of TIM (black line) and barley (light grey line) end of June to mid–October 2009, mid–May to end of October 2010 and mid–May to end of September 2011. (b) Ecosystem respiration (TER, black circles) and net ecosystem  $\text{CO}_2$  exchange (NEE, grey circles) from June 2009 until September 2011. (c) TER, below ground respiration (BR, blue squares) and soil respiration (SR, grey triangle) from May to October 2010 and May to September in 2011. (d) Methane ( $\text{CH}_4$ , grey diamonds) exchange from June 2009 until September 2011. The dashed black lines show the zero level.

AGR being the highest (Appendix Table A4). On a seasonal mean basis, RR accounted for 11% and 19%, SR for 30% and 31% and AGR for 58% and 50% of the TER, in 2010 and 2011, respectively.

Factors associated with vegetation, photosynthetically active radiation (PAR) and temperature were important in controlling the  $\text{CO}_2$  exchange of TIM. During May–September, TER correlated positively with barley height in 2009, air and soil temperatures in 2010 and with leaf area index and air temperature in 2011 (Appendix Table A5). Air temperature explained 32%, 42%



**Fig. 3.** Relationships between ecosystem respiration (TER) and air temperature ( $T_A$ ) and between gross primary productivity (GPP) and photosynthetically active radiation (PAR) on a mixture of timothy and meadow fescue (TIM). Measured TER (mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) was averaged with binned  $T_A$  (steps of 1°C) for (a) June–September 2009, (b) May–September 2010 and (c) May–September 2011. The respiration data were fitted with an exponential regression model in the form of  $TER = R_{10} \times Q_{10}^{(T_A/T_{10})}$ , where  $T_{10}$  is 10°C, fitted parameters are  $R_{10}$  (base respiration) and  $Q_{10}$  (temperature sensitivity). Measured GPP (mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) averaged with binned PAR (steps of 10 μmol m<sup>-2</sup> s<sup>-1</sup>) for (d) June–September 2009, (e) May–September 2010 and (f) May–September 2011. The GPP data were fitted with a rectangular hyperbolic model of the form of  $GPP = (GP_{max} \times PAR \times a) / (GP_{max} + PAR \times a)$ , where  $GP_{max}$  (μmol m<sup>-2</sup> s<sup>-1</sup>) is a theoretical maximum rate of photosynthesis at infinite PAR and  $a$  is apparent quantum yield. The fit results are given within the figure together with adjusted  $R^2$  of the regressions and number of bins ( $n$ ).

and 81% of the variation in the TER data for 2009, 2010 and 2011, respectively, when TER was binned with air temperature ( $T_A$ ) and fitted with an exponential regression model (Fig. 3a, b and c). The gross primary productivity (GPP) correlated positively with plant variables in each season, i.e. barley height ( $r_s = 0.755$ ,  $n = 64$ ,  $p < 0.05$ ) in June–September 2009, TIM height ( $r_s = 0.768$ ,  $n = 126$ ,  $p < 0.05$ ) in May–September 2010 and LAI ( $r_s = 0.750$ ,  $n = 124$ ,  $p < 0.05$ ) in May–September 2011. In addition, PAR explained 44%, 78% and 51% of the variation in the GPP in 2009, 2010 and 2011, respectively, when GPP was binned with PAR and fitted with a rectangular hyperbolic model (Fig. 3d, e and f).

We measured TER also from subplots with soil without vegetation (BARE). Although there was a clear seasonal variation in the soil temperature between summer and snow-covered seasons (Fig. 4a), the seasonal differences in TER were low (Fig. 4b). TER had a peak of

1500 mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> that occurred during the snow-free season. During May–September 2010, TER was linearly correlated with soil temperatures (Appendix Table A5). During the winter 2010–2011, the snow depth and CO<sub>2</sub> concentration in soil profile correlated with TER. Air temperature explained 45% and 25% of the variation in the data in 2010 and 2011, respectively, when TER data were binned to groups based on air temperature and fitted using an exponential regression model (Appendix Fig. A1). The fit was not statistically significant in 2009.

## Methane dynamics

Methane exchange varied between low uptake and emission on TIM (Fig. 2d) and BARE (Fig. 4c). The mean annual uptake was 140 mg CH<sub>4</sub> m<sup>-2</sup> and 120 mg CH<sub>4</sub> m<sup>-2</sup> on TIM and BARE, respectively (Table 2). On TIM,



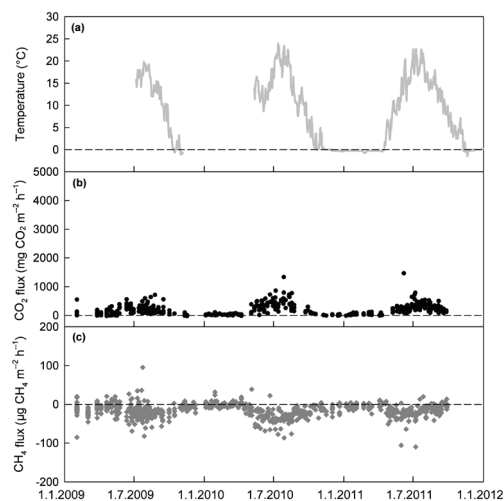
there were no significant correlations between the  $\text{CH}_4$  flux and environmental variables during May–September. However, the  $\text{CH}_4$  flux was positively correlated with the  $\text{CH}_4$  concentration at the 30 cm depth during the winter 2010–2011 ( $r_s = 0.593$ ,  $n = 18$ ,  $p < 0.05$ ). There were no significant correlations between the environmental variables and  $\text{CH}_4$  flux on BARE.

### Annual C budget and crop yield

The overall range of the annual TER varied between  $950 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$  (BARE in 2009) and  $4900 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$  (TIM in 2010, Table 2). On TIM, the GPP ranged from  $-3000 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$  (2009) to  $-5700 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$  (2011). The annual NEE was negative each year, showing that the cultivation system acted as a sink for  $\text{CO}_2$  throughout the studied years. During the three-year period (cumulative value), BARE released of  $3800 \text{ g CO}_2 \text{ m}^{-2}$  and the TIM was a sink of  $3000 \text{ g CO}_2 \text{ m}^{-2}$ . The annual  $\text{CO}_2 + \text{CH}_4$  budget as  $\text{CO}_2$ -equivalents of TIM and BARE was dominated by the  $\text{CO}_2$  exchange (Table 2).

TIM was harvested once in 2009 ( $6400 \text{ kg DW ha}^{-1}$ ) and twice in 2010 ( $13\,000 \text{ kg DW ha}^{-1}$ ) and 2011 ( $14\,000 \text{ kg DW ha}^{-1}$ ). The plant height development patterns of TIM were consistent with the crop removal and regrowth (Fig. 2a). In 2009, TIM was established with barley as a cover crop. The barley crop was taller than TIM (Fig. 2a).

As TIM represents a managed grassland, the fertilizer nitrogen use efficiency (NUE) and the sink strength were determined. Nitrogen use efficiency is the crop yield per kg of fertilizer



**Fig. 4.** Seasonal variation of the soil temperature, respiration and methane flux on treatment without vegetation (BARE). (a) Daily soil temperature ( $T_s$ , grey line) at 5 cm depth from July 2009 until end of 2011, (b) measured ecosystem respiration (TER, black circle) and (c) methane ( $\text{CH}_4$ , grey diamond) exchange from February 2009 until September 2011. The dashed black lines show the zero level.

applied ( $\text{kg DW ha}^{-1}$  per  $\text{kg N ha}^{-1}$ ). The overall NUE was  $80 \text{ kg DW kg N}^{-1}$ . The sink strength of TIM represents the net amount of  $\text{CO}_2$  taken up per  $\text{kg DW}$  of yield. The overall sink strength was  $0.9 \text{ kg CO}_2 \text{ kg DW}^{-1}$ .

## Discussion

### $\text{CO}_2$ exchange in the high latitudes

Among other things, climate change mitigation and adaption are contributing to an increased

**Table 2.** Annual ecosystem respiration (TER), gross primary productivity (GPP), net ecosystem  $\text{CO}_2$  exchange (NEE) and methane ( $\text{CH}_4$ ) uptake are shown for a mixture of timothy and meadow fescue (TIM). For soil without vegetation (BARE) annual TER and  $\text{CH}_4$  uptake are shown. Data are annual values with standard deviation ( $n = 3$ ).

|      | Year | TER<br>$\text{g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$ | GPP<br>$\text{g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$ | NEE<br>$\text{g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$ | $\text{CH}_4$<br>$\text{g CH}_4 \text{ m}^{-2} \text{ yr}^{-1}$ | $\text{CH}_4$<br>$\text{g CO}_2\text{-eq m}^{-2} \text{ yr}^{-1}$ |
|------|------|---|---|---|---|---|
| TIM  | 2009 | $1800 \pm 78$   | $-3000 \pm 870$                                       | $-1200 \pm 950$                                       | $-0.11 \pm 0.02$  | -3.1  |
|      | 2010 | $4900 \pm 730$  | $-5400 \pm 1500$                                      | $-560 \pm 1900$                                       | $-0.17 \pm 0.05$  | -4.7  |
|      | 2011 | $4400 \pm 320$  | $-5700 \pm 750$                                       | $-1300 \pm 890$                                       | $-0.13 \pm 0.03$  | -3.6  |
| BARE | 2009 | $950 \pm 110$   |   |   | $-0.12 \pm 0.01$  | -3.4  |
|      | 2010 | $1600 \pm 140$  |   |   | $-0.14 \pm 0.02$  | -3.8  |
|      | 2011 | $1300 \pm 230$  |   |   | $-0.10 \pm 0.03$  | -2.8  |

interest in reducing greenhouse gas emissions from agriculture and increasing the soil's capacity to store more carbon. However, there is a lot of uncertainty associated with the climate change and mitigation potential of agricultural soils (e.g., Crutzen *et al.* 2008, Creutzig *et al.* 2015, [www.4p1000.org](http://www.4p1000.org)). To estimate the overall atmospheric impacts of agricultural land-use and also bioenergy production, a robust life cycle analysis is needed. As a prerequisite, such analyses should include measurements of GHG exchange of cropping systems. Therefore, the aim of this study was to quantify and characterize the C exchange of perennial timothy and meadow fescue, a grassland ecosystem used for fodder of cattle and bioenergy in Finland. Our results show that this cropping system was an annual C sink. The mean CO<sub>2</sub> uptake was 1000 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> with GPP of -4700 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> and TER of 3700 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>. These results need to be assessed from the perspective of similar cropping systems adopted in the high latitude region. Therefore, we compiled published CO<sub>2</sub> fluxes from perennial and annual systems on both mineral and organic soils in northern Europe (Table 3). The main criterion for including a study in our analysis was that the study should report values of NEE, TER and GPP based on year-round measurements. Moreover, we did not include in this analysis any mulching or grazing studies nor systems without fertilizer. Including the present study, we compiled data from 22 cases which spanned across 54°N and 66°N latitudes. The cases in Table 3 are skewed more towards organic soils (82%) and perennial systems (64%) as the number of studies on mineral soils in this region is less. Annual NEE was available from 82%, GPP from 59% and TER from 77% of the studies selected. Perennial crops included buffalo grass (*Anthoxanthum odoratum*), festulolium (*Festulolium*), reed canary grass (*Phalaris arundinacea*), ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and different grass species and their mixtures such as timothy and meadow fescue. Annuals crops considered here are barley (*Hordeum vulgare*), wheat (*Triticum*), oat (*Avena sativa*) and potato (*Solanum tuberosum*).

The range of GPP values reported in the studies reviewed here varied from

-8000 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> on perennial tall fescue system on organic soil in Denmark (Kandel *et al.* 2017) to -1600 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> on perennial RCG system on a cut-away peatland in Estonia (Järveoja *et al.* 2016). Perennial RCG system on a cut-away peatland in Finland had the lowest TER (1800 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>, Shurpali *et al.* 2009) and annual whole crop system on organic soil in Germany had the highest (11 000 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>, Poyda *et al.* 2016). Mean annual GPP (-4700 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>) and TER (3700 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>) of TIM in this study (Table 2) are within in the range of the published values for different cropping systems in northern Europe (Table 3).

Net ecosystem CO<sub>2</sub> exchange (NEE) is the balance between the uptake of atmospheric CO<sub>2</sub> by vegetation and release of CO<sub>2</sub> through heterotrophic and autotrophic respiration. The CO<sub>2</sub> balance of a cropping system can vary from being a CO<sub>2</sub> sink or a source. As shown in Table 3, the range of NEE values varied from -2800 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> for perennial festulolium and tall fescue cropping systems on an organic soil in Denmark (Kandel *et al.* 2017) to 3700 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> on an annual whole crop silage system on organic soil in Germany (Poyda *et al.* 2016). These two studies reporting the extreme NEE values were carried out on a drained organic soil with similar C/N ratios (12) under similar climatic conditions (air temperature 8.4–9.0°C and precipitation 890–900 mm). The cropping systems had similar GPP values whereas the annual TER of the German system was about double of that of the Danish systems. At the Danish site, the low TER was associated with low temperatures during May to July and a high water table. At the German site, higher respiration occurred when the new crop was established, the water table was low and temperatures were high.

To understand if a certain pattern emerges, we grouped the data in Table 3 according to crop rotation (perennial vs annual) and soil type (organic vs mineral). The mean NEE from mineral soils was -570 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> with annual crops and -1000 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> with perennial systems. For organic soils, the mean NEE value was 1000 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> on annual systems and 410 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup> on perennial systems.

**Table 3.** Annual net ecosystem CO<sub>2</sub> exchange (NEE, g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>), gross primary productivity (GPP, g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>) and ecosystem respiration (TER, g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>) of annual and perennial cultivation systems located in northern Europe (54–66°N). Table also includes study period, soil type (organic (O), mineral (M)), plant type (festulolium (F), reed canary grass (RCG), spring barley (SB), tall fescue (TF), winter barley (WB), whole crop silage (WCS), winter wheat (WW), oat (O), potato (P), buffalo grass (BF), ryegrass (RG), barley with timothy and meadow fescue (B+TMF)), crop cycle (annual (A), perennial (P)), average yield (kg DW ha<sup>-1</sup>), average nitrogen (N) added as fertilizer (kg N ha<sup>-1</sup>), average fertilizer nitrogen use efficiency (NUE, kg DW kg N<sup>-1</sup>) by the system and flux measurement method (eddy covariance (EC), chamber methods (CB)). Dashed lines are unavailable data.

| Area | Period    | Soil | Plant   | Cycle | Yield  | N   | NUE | Method | NEE   | GPP   | TER    | Reference            |
|------|-----------|------|---------|-------|--------|-----|-----|--------|-------|-------|--------|----------------------|
| DK   | 2015      | O    | F       | P     | 18 000 | 160 | 110 | CB     | -2800 | -7300 | 5100   | Kandel et al. 2017   |
| DK   | 2015      | O    | TF      | P     | 16 000 | 160 | 100 | CB     | -2800 | -8000 | 4800   | Kandel et al. 2017   |
| DK   | 2004      | M    | B+grass | P     | –      | 180 | –   | EC     | -1100 | -6900 | 5700   | Gilmanov et al. 2007 |
| FI   | 2009–2011 | M    | TIM     | P     | 11 000 | 150 | 80  | CB     | -1000 | -4700 | 3700   | present study        |
| DK   | 2004–2007 | O    | WW      | A     | –      | –   | –   | EC     | -1000 | -4600 | 3600   | Kutsch et al. 2010   |
| FI   | 2010–2011 | M    | RCG     | P     | 6500   | 73  | 88  | EC     | -950  | -4600 | 3700   | Lind et al. 2016     |
| DK   | 2009–2014 | M    | WB/SB   | A     | –      | –   | –   | EC     | -570  | -4100 | 3500   | Jensen et al. 2017   |
| FI   | 2004–2007 | O    | RCG     | P     | 2900   | 60  | 49  | EC     | -370  | -2200 | 1800   | Shurpali et al. 2009 |
| DK   | 2011      | O    | SB      | A     | 10 000 | 120 | 85  | CB     | -150  | -4900 | 4700   | Kandel et al. 2013   |
| DK   | 2011      | O    | RCG     | P     | 12 000 | 60  | 200 | CB     | 250   | -6700 | 6900   | Kandel et al. 2013   |
| EE   | 2014      | O    | RCG     | P     | 4900   | 72  | 68  | CB     | 290   | -1600 | 1900   | Järveoja et al. 2016 |
| FI   | 2001–2002 | O    | B+TMF   | P     | 5900   | 92  | 67  | EC     | 530   | –     | –      | Lohila et al. 2004   |
| FI   | 2000      | O    | grass   | P     | –      | –   | –   | CB     | 1500  | –     | –      | Maljanen et al. 2004 |
| FI   | 1997      | O    | SB      | A     | 9200   | 60  | 150 | CB     | 1500  | –     | –      | Maljanen et al. 2001 |
| DE   | 2007–2008 | O    | BF+RG   | P     | –      | 120 | –   | CB     | 1700  | -6900 | 8600   | Beetz et al. 2013    |
| FI   | 1997      | O    | grass   | P     | 5600   | 100 | 56  | CB     | 2800  | –     | –      | Maljanen et al. 2001 |
| FI   | 2000      | O    | SB      | P     | –      | 100 | –   | CB     | 3000  | –     | –      | Maljanen et al. 2004 |
| DE   | 2012–2013 | O    | WCS     | A     | 11 000 | 150 | 76  | CB     | 3700  | -6900 | 11 000 | Poyda et al. 2016    |
| FI   | 1991–1992 | O    | grass   | P     | –      | –   | –   | CB     | –     | –     | 2200   | Nykänen et al. 1995  |
| DK   | 2014      | O    | P+SB    | A     | 8 000  | 120 | 66  | CB     | –     | –     | 4700   | Kandel et al. 2018   |
| DK   | 2014      | O    | O+P     | A     | 13 000 | 84  | 150 | CB     | –     | –     | 4500   | Kandel et al. 2018   |
| DK   | 2014      | O    | O+SB    | A     | 15 000 | –   | –   | CB     | –     | –     | 6000   | Kandel et al. 2018   |

It seems that perennial systems have a higher capacity to take up  $\text{CO}_2$  than the annual systems. This was more evident for mineral soils than organic soils. However, when interpreting these results, it should be noted that the number of studies on mineral soils was low ( $n = 1$  for annuals and  $n = 2$  for perennials). The mean annual NEE ( $-1000 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$ ) of TIM in this study (Table 2) is within the upper range of the NEE values for cropping systems in northern Europe (Table 3).

The  $\text{CO}_2$  exchange studies of perennial cropping systems summarized in Table 3 were mostly carried out after the systems were well established. Only three studies reported  $\text{CO}_2$  exchange from the beginning of the cultivation (present study; Lohila *et al.* 2004; Lind *et al.* 2016). In our study, the GPP and TER values in the establishment year differed from the values obtained in later years (Table 2). The effect of crop type on  $\text{CO}_2$  exchange during the establishment year varies. Net ecosystem  $\text{CO}_2$  exchange of reed canary grass (RCG) cultivated on the same field as TIM here was determined from July 2009 onwards during the establishment year (Lind *et al.* 2016). To fill the data gap in Lind *et al.* (2016), we used the results from BARE plots as the RCG site then represented a bare site. The mean annual NEE of RCG estimated this way was  $-530 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$  which is half of that for TIM. The main differences in the mean annual NEE arise from the C sink dynamics in TIM and RCG in 2009. The grass mixture of timothy and meadow fescue was sown in June 2009 with barley as a cover crop. Therefore, NEE measured prior to the harvest in 2009 represents the combined NEE of these three crops. Of these, barley had the most vigorous growth and it reached its maximum annual growth prior to the harvest in August 2009 while timothy and meadow fescue mixture reached two-thirds of its potential annual growth (Fig. 2b). On the contrary, as a slowly establishing grass species (Casler and Kallenbach 2007), the reed canary grass reached about a third of its maximum growth in 2009 (data not shown).

Mean annual TER ( $1300 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$ ) of BARE in this study (Table 2) was 65% lower than that of TIM. As BARE plots were devoid of any vegetation, the TER from these plots repre-

sented heterotrophic respiration (soil microbial respiration) only, whereas the TER from TIM included both heterotrophic and autotrophic respiration components. On average, the heterotrophic respiration from TIM contributed about a third of its TER (Appendix Table A4). Using this partitioning factor, the annual heterotrophic respiration for TIM is estimated to be about  $1200 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$  — a value that is comparable to  $\text{CO}_2$  loss from BARE. Based on this, it is possible that there was no priming effect on TIM due to the fresh biomass.

### Methane in the annual C budget

Methane is part of the C budget of a cropping system. The conditions at our study site with well-drained mineral soil were not favorable for methanogenesis but allowed methane oxidation to occur, and hence, the site acted as a sink for atmospheric methane (Table 2). Low annual  $\text{CH}_4$  uptake rates are typical for grass systems on mineral soils in Finland ( $-72 \text{ mg CH}_4 \text{ m}^{-2} \text{ yr}^{-1}$  by Syväsalö *et al.* (2006) and  $-36 \text{ mg CH}_4 \text{ m}^{-2} \text{ yr}^{-1}$  by Regina *et al.* (2007),  $-60 \text{ mg CH}_4 \text{ m}^{-2} \text{ yr}^{-1}$  by Maljanen *et al.* (2012)). When compared with the net annual  $\text{CO}_2$  uptake, the contribution of  $\text{CH}_4$  was low. The  $\text{CH}_4$  uptake expressed as  $\text{CO}_2$  equivalents accounted for less than 1% of the net annual budgets.

### Nitrogen use efficiency of TIM and other crops

To assess how efficient the perennial cropping system adopted in this study was in utilizing the applied N, we determined its fertilizer nitrogen use efficiency (NUE) and compared it with that of other cropping systems from other studies. The NUE of TIM was  $80 \text{ kg DW kg}^{-1} \text{ N}$  and it is within the range of NUE values estimated for other systems listed in Table 3. The highest NUE was from an autumn-harvested RCG cropping system on organic agricultural field in Denmark ( $200 \text{ kg DW kg}^{-1} \text{ N}$ , Kandel *et al.* 2013) and the lowest on a spring-harvested RCG cropping system on cut-away peatland in Finland ( $49 \text{ kg DW kg}^{-1} \text{ N}$ , Shurpali *et al.* 2009). When

including all three years in this study, the NUE of RCG was lower (63 kg DW kg<sup>-1</sup> N) than that of TIM. This shows, that under similar growing conditions, TIM as a cropping system utilizes more effectively the applied N than RCG.

## Future studies

This study covers the crop establishment phase of TIM cropping system on mineral soil. However, it does not account for the entire rotation period of the system. In Finland, timothy and meadow fescue cropping systems are renewed every three to four years (Virkajärvi *et al.* 2015) due to decreasing yields caused by winter damages and increased number of weeds. Owing to a short rotation period, TIM system needs to be tilled for the re-establishment after the rotation cycle. This increases C release from the soil to the atmosphere. Therefore, studies should cover not only the entire crop rotation cycle but also the re-establishment phase. Timothy and meadow fescue grasslands are a preferred forage system in the study region as they support livestock production and dairy industries, the mainstay of the regional economy. Crop management options, such as direct drilling of TIM (no-tillage during the crop re-establishment after a rotation period), are increasingly being practiced as a means of controlling GHG emissions from soils (Mangalassery *et al.* 2014). However, more long-term studies that compare the advantages of tillage vs no-tillage (direct drilling) of grasslands under boreal environmental conditions are needed.

To mitigate climate change and to develop a robust agricultural and bioenergy policies, an annual GHG balance of biomass production is needed. Here, we assessed the annual CO<sub>2</sub> and CH<sub>4</sub> budget of timothy and meadow fescue mixture on a mineral soil under the boreal climate. We further characterized the factors controlling the CO<sub>2</sub> and CH<sub>4</sub> exchange of this cultivation system. Our study site was an annual sink for these C gases. The NEE reported here was similar to the NEE values of perennial cropping systems on mineral soils and higher when compared with that of annual cropping systems in northern Europe. The NUE, determined as a ratio of the

amount of nitrogen applied to crop yield, was within the range of the values reported for other cropping systems in northern Europe. We found that the NUE of timothy and meadow fescue mixture was higher than that of reed canary grass, a perennial cultivation system in a companion study (Lind *et al.* 2016). The results here support the growing body of literature highlighting the benefits of perennial agriculture. Beyond yield levels, the perennial agriculture increases sustainability and adds value to the functions of the ecosystem processes and services

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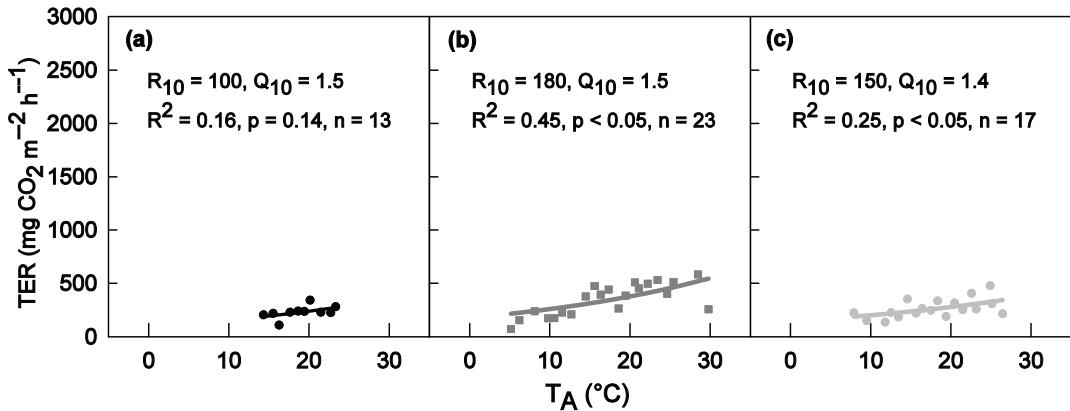
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## Appendix



**Fig. A1.** Relationship between ecosystem respiration (TER) and air temperature ( $T_A$ ) on soil without vegetation (BARE). Measured TER ( $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) was averaged with binned  $T_A$  (steps of  $1^\circ\text{C}$ ) for (a) June–September 2009, (b) May–September 2010 and (c) May–September 2011. The data were fitted with an exponential regression model in the form of  $\text{TER} = R_{10} \times Q_{10}^{(T_A/T_{10})}$ , where  $T_{10}$  is  $10^\circ\text{C}$ , fitted parameters are  $R_{10}$  (base respiration) and  $Q_{10}$  (temperature sensitivity). The fit results are given within the figure together with adjusted  $R^2$  of the regressions and number of bins ( $n$ ).

**Table A1.** Fit results of the respiration models. The ecosystem respiration (TER) was modelled for mixture of timothy and meadow fescue (TIM) and soil without vegetation (BARE) using an exponential regression model in the form of  $\text{TER} = R_{10} \times Q_{10}^{(T_A/T_{10})}$ , where  $T_A$  is the measured air temperature,  $T_{10}$  is  $10^\circ\text{C}$ , fitted parameters are  $R_{10}$  (base respiration) and  $Q_{10}$  (temperature sensitivity). The ranges of  $R_{10}$ ,  $Q_{10}$  and the  $R^2$  of the fit are shown for each of the season and treatments.

| Season              | Treatment | $R_{10}$ | $Q_{10}$ | $R^2$     |
|---------------------|-----------|----------|----------|-----------|
| June–September 2009 | TIM       | 110–220  | 1.7–2.7  | 0.05–0.06 |
|                     | BARE      | 90–120   | 1.2–1.7  | 0.01–0.05 |
| May–September 2010  | TIM       | 510–640  | 1.5–1.6  | 0.15–0.44 |
|                     | BARE      | 130–240  | 1.2–1.8  | 0.15–0.37 |
| May–September 2011  | TIM       | 280–350  | 1.9–2.3  | 0.22–0.40 |
|                     | BARE      | 100–170  | 1.4–1.6  | 0.03–0.18 |

**Table A2.** Fit results of gross primary production models. The gross primary productivity (GPP) for mixture of timothy and meadow fescue (TIM) was modelled using an exponential regression model in form of  $\text{GPP} = (\text{GP}_{\text{max}} \times \text{PAR} \times \alpha) / (\text{GP}_{\text{max}} + \text{PAR} \times \alpha)$  as Eq. 2 and in form of  $\text{GPP} = (\text{GP}_{\text{max}} \times \text{PAR} \times \alpha) / (\text{GP}_{\text{max}} + \text{PAR} \times \alpha) + (c + d \times \text{height})$  as Eq. 3. The range of  $\text{GP}_{\text{max}}$  ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ , a theoretical maximum rate of photosynthesis at infinite PAR),  $\alpha$  (apparent quantum yield),  $c$  and  $d$  (scaling parameters for plant height) and also  $R^2$  of the fit are shown for each of the season and both of the used equations.

| Season        | Treatment   | $\text{GP}_{\text{max}}$<br>(max/min) | $\alpha$<br>(max/min) | $c$<br>(max/min) | $d$<br>(max/min) | $R^2$<br>(max/min) |
|---------------|-------------|---------------------------------------|-----------------------|------------------|------------------|--------------------|
| Jun–Sep. 2009 | TIM (Eq. 2) | –3400 / –8200                         | –5.9 / –8.1           |                  |                  | 0.11–0.16          |
|               | TIM (Eq. 3) | –4200 / –9200                         | –3.2 / –27            | 80–3300          | –68 / –72        | 0.34–0.55          |
| May–Sep. 2010 | TIM (Eq. 2) | –4300 / –7600                         | –8.6 / –23            |                  |                  | 0.06–0.11          |
|               | TIM (Eq. 3) | –6600 / –11000                        | –4.0 / –6.5           | 810–1800         | –51 / –69        | 0.48–0.60          |
| May–Sep. 2011 | TIM (Eq. 2) | –6200 / –7600                         | –8.9 / –11            |                  |                  | 0.11–0.37          |
|               | TIM (Eq. 3) | –7700 / –12000                        | –5.5 / –102           | 980–8700         | –44 / –55        | 0.42–0.71          |

**Table A3.** Monthly and annual air temperature ( $T_A$ , °C) during 2009–2011 and precipitation sums ( $P$ , mm) at the study site.

|       | Year | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. | Aug. | Sep. | Oct. | Nov. | Dec. | Annual |
|-------|------|------|------|------|------|-----|------|------|------|------|------|------|------|--------|
| $T_A$ | 2009 | −8.3 | −8.6 | −3.7 | 1.8  | 11  | 11   | 16   | 15   | 11   | 1.1  | 0.7  | −8.8 | 3.4    |
|       | 2010 | −17  | −13  | −5.2 | 3.3  | 11  | 13   | 21   | 16   | 10   | 3.4  | −4.8 | −14  | 2.0    |
|       | 2011 | −9.1 | −17  | −3.4 | 4.5  | 9.5 | 16   | 19   | 15   | 11   | 5.3  | 1.7  | 0.2  | 4.4    |
| $P$   | 2009 | 33   | 25   | 10   | 21   | 29  | 36   | 51   | 66   | 20   | 51   | 54   | 26   | 420    |
|       | 2010 | 21   | 48   | 58   | 25   | 54  | 74   | 10   | 58   | 60   | 38   | 39   | 31   | 520    |
|       | 2011 | 73   | 24   | 28   | 24   | 47  | 48   | 142  | 84   | 67   | 43   | 14   | 77   | 670    |

**Table A4.** Partitioning of the ecosystem respiration to root respiration (RR), soil respiration (SR) and aboveground respiration (AGR) in mg CO<sub>2</sub> m<sup>−2</sup> h<sup>−1</sup> on a mixture of timothy and meadow fescue (TIM). Data are means with standard deviation.

| Year | RR        | SR        | AGR       |
|------|-----------|-----------|-----------|
| 2010 | 150 ± 140 | 360 ± 84  | 680 ± 370 |
| 2011 | 210 ± 150 | 350 ± 100 | 580 ± 270 |

**Table A5.** The Spearman's rank correlation coefficients between measured ecosystem respiration (TER) and environmental variables on the mixture of timothy and meadow fescue (TIM) and soil without vegetation (BARE) when statistically significant correlations were observed. Environmental variables are barley height (Barley), air temperature ( $T_A$ , °C), soil temperature at 0 cm, 2 cm, 5 cm, 10 cm and 20 cm depths ( $T_S$ , °C), leaf area index (LAI), snow depth and CO<sub>2</sub> concentration in the soil profile at 5 cm and 30 cm depths. The correlation coefficients are given for those occasions when the coefficient was higher than 0.6 and the correlation was statistically significant ( $p < 0.05$ ).

|      | Jun.–Sep. 2009              | May–Sep. 2010   | Winter 2010–2011  | May–Sep. 2011  |
|------|-----------------------------|---|---|--|
| TIM  | Barley (0.711**, $n = 72$ ) | $T_A$ (0.643**, $n = 147$ )<br>$T_S$ , 2 cm (0.616**, $n = 147$ )<br>$T_S$ , 5 cm (0.619**, $n = 147$ )<br>$T_S$ , 10 cm (0.603**, $n = 147$ )  | CO <sub>2</sub> , 30cm (−0.777**, $n = 12$ )                                  | $T_A$ (0.608**, $n = 171$ )<br>LAI (0.632**, $n = 124$ ) |
| BARE |                             | $T_S$ , 0 cm (0.631**, $n = 88$ )<br>$T_S$ , 2 cm (0.633**, $n = 88$ )<br>$T_S$ , 5 cm (0.634**, $n = 88$ )<br>$T_S$ , 10 cm (0.617**, $n = 88$ )<br>$T_S$ , 20 cm (0.602**, $n = 88$ ) | Snow depth (0.619**, $n = 69$ )<br>CO <sub>2</sub> , 5 cm (0.520*, $n = 19$ ) |  |

\* Significance at the 0.05 level

\*\* Significance at the 0.01 level