Study areas

This study was carried out on soil samples from 150 permanent grasslands in three study regions: The Schorfheide‑Chorin, an UNESCO biosphere reserve located in the north‑east of Germany, the national park Hainich‑Dün and its surrounding in Central Germany, and the UNESCO biosphere area Schwäbische Alb in south‑west Germany ([Fischer et al. (2010)](#_ENREF_6); www.biodiversity-exploratories.de). Each study region has a specific climate and parent material (Table 1). The mean annual temperature (MAT) ranges from 6.5 °C in the Schwäbische Alb to 8 °C in the Schorfheide‑Chorin. The mean annual precipitation (MAP) increases in the order Schorfheide‑Chorin, Hainich‑Dün and Schwäbische Alb from 520 to 963 mm. In the Schorfheide‑Chorin a layer of aeolian and fluvial sand covers the underlying glacial till. The parent material in the Hainich‑Dün is loess over Triassic shell lime stone and in the Schwäbische Alb soils developed on Jurassic lime stone. The main WRB soil groups are Cambisols, Luvisols and Histosols in the Schorfheide Chorin, Cambisols, Luvisols and Stagnosols in the Hainich-Dün, and Cambisols and Leptsols in the Schwäbische Alb ([IUSS-Working-Group-WRB 2006](#_ENREF_9)).

Land use

In 2011, all study plots had been used as grassland for more than 20 years. Since 2006, data on the grassland management were collected annually for all plots with a questionnaire which was distributed to all farmers. They reported on the number of animals (cows, horses or sheeps) and the grazing duration. This enabled the calculation of the grazing intensity (G). Farmers also reported the annual mowing frequency (M) and the annual nitrogen fertilisation (F). We used land-use information from 2006 to 2010 for each plot and calculated averages across years for G, M and F. The average values for G, M and F were standardized by the respective means of G, M and F in the three study regions. Land use intensity (LUI) for each grassland plot was quantified by the sum of the standardized G, M and F ([Blüthgen et al. 2012](#_ENREF_2)).

Soil sampling

We sampled the surface soil (0 10 cm depth) at 14 locations per plot along two 18 m transects. The distance between sampling points was 3 m. Mineral soil samples were taken using a split tube auger, 40 cm long and 5 cm wide (Eijkelkamp, Giesbeek, The Netherlands). A composite soil sample was prepared by mixing all soil samples from the same plot. Each composite sample was homogenized, sieved (<2 mm), weighed and split into three subsamples. One aliquot was air-dried (at 40°C), another one was kept field moist at a temperature of +4°C and a third one was frozen at -20°C.

Basic soil analyses

A subsample (10 g) of the field moist soil was used to determine gravimetric water content. Air-dried soil was used for the determination of soil pH, soil texture and CN concentrations. We measured the soil pH in the supernatant of 1:2.5 mixture of soil and 0.01M CaCl2. Soil texture was determined after removal of soil organic matter by the pipette method (Schlichting et al. 1995). Total carbon (TC) and nitrogen (N) contents were analyzed on ground subsamples by dry combustion in a CN analyzer “Vario Max” (Elementar Analysensysteme GmbH, Hanau, Germany). Inorganic carbon (IC) was determined after combustion of organic carbon in a muffle furnance (450°C for 16 h). The soil organic carbon (SOC) equals the difference between TC and IC.

Microbial biomass carbon

Microbial biomass C was determined on frozen samples by chloroform-fumigation-extraction (Vance et al. 1987). Field moist soil (10 g) was fumigated for 24 h with ethanol-free chloroform in a desiccator under vacuum. After removal of the chloroform, dissolved organic carbon (DOC) was extracted by adding 40 ml of a 0.5 M K2SO4-solution (1:4 w/v soil/extractant ratio). Subsequently, samples were shaken for 30 min at 250 rev min-1 on a horizontal shaker and centrifuged for 30 min at 4422 g. A second sample of field moist soil (10 g) was treated in the same way but without fumigation. Dissolved OC in the supernatants was measured with a DOC analyzer (Multi N/C 2100S, Analytik Jena, Jena, Germany). The difference between dissolved C in the fumigated and non-fumigated samples is the chloroform labile C pool.

Soil Incubations

We incubated sieved (<2mm) field-moist soil at a constant temperature of 20°C. All samples were moistened to a standardised water holding capacity (WHC) of 60%. This allowed us excluding the water content as a variable in subsequent analyses. Depending on initial C concentrations, 70 to 250 g soil were filled in 250 ml beaker glasses and placed in 1000 ml glass jars with airtight lids and two stopcocks. The relative humidity in the CO2 free air was adapted to an ambient level by adding 5 mL of deionised water on the bottom of the glass jars. After a preincubation period of 4 days, the jar headspace was purged with CO2 free air. Soil samples were then incubated for 14 days. The CO2 release from soil was measured after 1, 3, 7 and 14 days on 1-2 ml aliquots of headspace air by a LI-6262 CO2/H2O infrared gas analyzer (LI-COR-Environmental, Lincoln, Nebraska USA). Fluxes reported in this study are calculated using the total amount of CO2 evolved after an incubation period of 14 days.