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# Relationships between greenhouse gas emissions and cultivable bacterial populations in conventional, organic and long-term grass plots as affected by environmental variables and disturbances

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Daily dynamics of greenhouse gas (GHG) emissions and cultivable bacterial populations have rarely been examined. The objectives were: (1) to investigate if dynamics of GHG emissions can be described by harmonics and are related to those of cultivable bacteria after soil disturbances in three grassland management systems; (2) to determine to which extent daily GHG emissions are related to environmental variables rather than disturbance events in two climate zones; and (3) to investigate differences in GHG emissions between organic and conventional tilled grassland versus no-till long-term grassland systems (OG, CG and LG, respectively). In replicated field experiments with OG, CG, and LG plots in the Netherlands and Russia, GHG ($CO_2$, $N_2O$ and $CH_4$) emissions and cultivable bacterial populations were measured daily during two one-month periods at each location. Tillage, fertilization, biomass incorporation and irrigation were considered disturbances. The dynamics were subjected to harmonics, cross-correlation, and canonical correspondence analyses (CCA). The dynamics of cultivable bacterial populations and GHG fluxes rarely reflected autonomous growth and death cycles of bacteria after a disturbance due to the overarching influences of environmental conditions, especially in spring. Thus, GHG emissions were influenced more by weather variables than by agronomic disturbances. This was confirmed by CCA. Cultivable bacterial populations were cross correlated with $CO_2$ fluxes and sometimes $N_2O$ emissions, but generally not with $CH_4$ fluxes. Average cultivable bacterial populations and $CO_2$ emissions were highest in OG and lowest in LG; $N_2O$ emissions were mostly highest in CG and lowest in LG; and $CH_4$ fluxes were frequently highest in OG and lowest in LG. Thus, although bacteria and GHG peaks were induced by disturbances, sometimes followed by autonomous oscillations due to growth and death cycles and associated cycles in nutrient and oxygen availability, the dynamics were mainly affected by environmental variables and long-term management, with the smallest GHG emissions from LG plots.


## 1. Introduction

Emissions of the greenhouse gases (GHG) nitrous oxide ($N_2O$), carbon dioxide ($CO_2$), and methane ($CH_4$) have received considerable attention in recent years in connection with observed and predicted global climate change. All three gases contribute to global

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warming, but the $CO_2$ equivalent effects of $N_2O$ and $CH_4$ on climate change are considered to be 300 and 36 times that of $CO_2$ over a period of 100 years (https://www.epa.gov/ghgemissions/). Agriculture is responsible for about 10% of GHG emissions in Canada, the USA and the EU, and 24% worldwide (EUROSTAT, 2016; Grant et al., 2004; https://www.epa.gov/ghgemissions/). Total annual GHG emissions from agriculture were 5335 Mt $CO_2$ eq in 2011 (Tubiello et al., 2014). $N_2O$ and $CH_4$ contribute to agricultural emissions in a major way, primarily through crop fertilization, tillage, and bacterial decomposition of manure and crop residues, as well as enteric fermentation in livestock (Tubiello et al., 2014). Europe is responsible for 12% of all agricultural emissions, but there is tremendous country-to-country variation due to differences in climate, livestock, crops and farming practices (EUROSTAT, 2016).

GHG emissions from agricultural land depend on the type of farming system, crops grown, and their production practices (Knudsen et al., 2014). The emissions are generally greater in tilled cultivated land than in no-till agricultural land or permanent grasslands (Ball et al., 2014; Jabro et al., 2008; Kurganova et al., 2007; Lognoul et al., 2017; Oorts et al., 2007; Shahidi et al., 2014). In agricultural areas where tillage is practiced, GHG emissions per unit area are often lower in organic (ORG) than in conventional (CONV) farms (Benoit et al., 2015; Knudsen et al., 2014; Skinner et al., 2014; Trimpler et al., 2016; Zhang et al., 2016), although there are exceptions to this rule (Bos et al., 2014; Nagano et al., 2012). GHG emissions from grassland compared to annual cropland primarily depend on fertilization and the number of years in grass (Brozyna et al., 2013). Grass-legume mixtures require less fertilizer than most crops, but in some areas, manure and fertilizer may be applied on mixed grassland too, potentially resulting in large $N_2O$ emissions (Schils et al., 2008). Turning under a grass-clover mixture results in a sharp peak in $N_2O$ emission, even without manure or fertilizer (Brozyna et al., 2013).

Long-term grassland (LG) or pasture soils are often considered more diverse and healthier than tilled arable soils (Lienhard et al., 2013), but the quality of ORG arable soils can approach that of long-term grassland or pasture soils. ORG farming systems generally have longer rotations than CONV systems, sometimes including grass (or grass-legume) leys in the rotation. The difference in management generally results in higher microbial densities and diversity (van Bruggen et al., 2006; van Diepeningen et al., 2006), and in improved soil health in ORG compared to CONV systems (Senechkin et al., 2014; van Bruggen et al., 2015; van Bruggen and Semenov, 2015; van Diepeningen et al., 2006). Healthy soils are characterized by greater resilience and resistance of microbial communities to disturbances, evidenced by enhanced internal nutrient cycling and dampened oscillations in cultivable and total bacterial populations in response to a disturbance (van Bruggen et al., 2006; van Bruggen et al., 2015; Zelenev et al., 2000, 2006). The oscillations in bacterial populations have periods of about 3–5 days, and have been attributed to growth and death cycles in response to carbon availability, or to alternations in micro-scale aerobic and anaerobic conditions (van Bruggen et al., 2006).

The alternations in carbon and oxygen availability and microbial populations is likely accompanied by oscillations in GHG emissions (Semenov et al., 2013; Zelenev et al., 2006), which are dependent on microbial processes (Oertel et al., 2016). For example, $CO_2$ is released mainly as a by-product of aerobic (or anaerobic) decomposition of organic compounds, $N_2O$ is emitted mainly as a result of denitrification under anaerobic conditions (Ball et al., 1999), and $CH_4$ is primarily produced by methanogenic bacteria under anaerobic conditions and emitted from soil when aerobic conditions return (Dutaur and Verchot, 2007; Le Mer and Roger, 2001; Oertel et al., 2016). Under controlled environmental conditions, associations between the oscillations in GHG emissions and cultivable bacterial populations have been documented (He et al., 2017).

However, under natural environmental conditions, fluctuations in GHG emissions from soil are affected by daily temperature variations (Maljanen et al., 2002; Oorts et al., 2007; Savage et al., 2014; Semenov et al., 2004), as well as by disturbances from rainfall events (Maljanen et al., 2002; Oorts et al., 2007; Savage et al., 2014), drying-rewetting or freezing-thawing cycles (Priemé and Christensen, 2001), and from soil tillage (Teixeira et al., 2013). These disturbances result in peaks in GHG emissions, sometimes followed by autonomous fluctuations that do not reflect diel variations in temperature or moisture, but have periods of several days. However, rarely have the daily dynamics of all three GHGs ($CO_2$, $N_2O$ and $CH_4$) been measured simultaneously under field conditions, together with those of cultivable bacterial populations. So far, these dynamics were compared for ORG and CONV soils without crops or with arable crops (He et al., 2017), but not for short- and long-term grasslands in different climates.

Harmonics analysis of daily measurements of cultivable bacterial populations and GHG emissions demonstrated the occurrence of regular oscillations after a disturbance by soil amendment under controlled environmental conditions, with significant cross correlations (Semenov et al., 2013). The amplitudes of the ratios of the measurements in amended over nonamended soils were smaller in ORG than in CONV soils (van Bruggen et al., 2015), indicating that the ORG soils might be healthier (van Bruggen and Semenov, 2015). However, under field conditions these relations were less clear-cut (Semenov et al., 2013).

Considering the paucity of temporal data on the relations between daily GHG emissions and bacterial populations, especially in tilled short-term grasslands and long-term grasslands, as related to daily climate data, this research was aimed at comparing the short-term dynamics of GHG emissions and cultivable bacterial populations in ORG and CONV versus LG soils in two different temperate climates (a maritime and continental climate). Specific objectives were: (1) to investigate if the dynamics of daily emissions of $CO_2$, $N_2O$ and $CH_4$ can be described by harmonics and are related to those of cultivable bacteria in three grassland management systems at two locations; (2) to determine if disturbance events or environmental and soil variables were the drivers of fluctuations in daily GHG emissions in three grassland management systems in two climate zones; and (3) to determine if there are significant differences in GHG emissions between ORG and CONV tilled grassland versus no-till LG systems. To attain these objectives, two similar experiments were set up with three grassland systems at two locations, in the Netherlands with a maritime climate and in Russia with a continental climate. Daily measurements of $CO_2$, $N_2O$, $CH_4$ emissions, as well as cultivable bacteria on C-rich and C-poor nutrient media, and temperature and soil moisture, were made in early season and late season at both locations.

## 2. Materials and methods

### 2.1. Site descriptions

Two similar field experiments were carried out at Wageningen University, the Netherlands, and at the Institute of Physicochemical and Biological Problems in Soil Science in Pushchino, Russia.

Wageningen is located in the center of the Netherlands (52.0° N, 5.67° E). The climate is a temperate maritime climate with warm summers and temperate winters, and an annual rainfall of 780 mm. The average annual temperature is 9.4°C. The experiment was carried out in 2005/2006 at the experimental organic (ORG) farm the Droevendaal (converted in 2002 and certified in 2004) with an 8-year rotation, and at an adjacent conventionally (CONV) managed