Chapter 9

Greenhouse Gases Production from Some Crops Growing Under Greenhouse Conditions

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Additional information is available at the end of the chapter

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Abstract

Greenhouse gases, such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), play an important role in global climate change. For example, CO_2 production occurs as a result of the seasonal cycles of the biotic processes of photosynthesis and respiration, as well as through anthropogenic activities and abiotic processes such as the burning of fossil fuels. Many activities, such as Agribusiness (the production of crops and animals for food) create greenhouse gases. Our research group has studied several soil treatments such as wastewater, wastewater sludge, vermicomposting, and urea among others, in order to study the effects of soil treatments on the production of greenhouse gases (CO_2, N_2O, and CH_4) in several cultivars, but mainly in maize, sunflower and the common bean. The principal aim of this chapter is to show how these greenhouse gases are affected by the type of treatment, the properties of the soil, and the cultivar in question. We also look at which processes are involved in the production of CO_2, N_2O, and CH_4 from cultivated soil. We present a review of several experiments carried out under *in vitro* or greenhouse conditions.

Keywords: Greenhouse gases production, wastewater sludge, fertilizers, treatments

1. Introduction

Global food demand is increasing rapidly, while the associated potential negative environmental impacts are also growing. Land clearance, the intensive use of existing croplands, inadequate agricultural management systems, and soil pollution could all contribute to an increase in the production of greenhouse gases (GHG). Understanding the future environmental impacts of global crop production, while at the same time achieving greater yields with lower impacts, requires quantitative assessments of future crop demand and an understanding of how different production practices affect yields and environmental variables [1].

It is well known that crop management systems, the quality of the soil and the weathering conditions are just some of the factors used in order to assess production of GHG. Therefore, an understanding of the future environmental impacts of crop production is essential in order to achieve greater crop yields without decreasing the quality of the environment and social welfare. Additionally, Tilman [2] reported that the recent intensification of agriculture, coupled with the prospect of even further intensification in the future, will have major detrimental impacts on the world's ecosystems.

Agriculture is rightly recognized as a source of GHG production, with concomitant opportunities for its mitigation. In fact, agricultural soils can constitute either a net source or sink of the three principal GHG [3]. Soil management practices can influence GHG flux by changing at least one of the following soil properties and its associated management: 1) The soil climate (temperature and water content); 2) The physicochemical environment of the soil; 3) The soil's microorganisms (diversity and abundance); 4) The amount and chemical composition of organic or mineral fertilizers applied to the soil; and 5) Pesticides might have a strong effect on the soil microbiota (type and amount). Even a minimal change in one or more of the properties described above could control the rate and extent of GHG production and also affect the aeration and diffusion of these gases.

The objective of this chapter is to discuss how the soil production rate of GHG is affected by treatment type, soil properties, and cultivar. This review will also discuss which processes are involved in the production of CO_2, N_2O, and CH_4 when crops are grown under *in vitro* or greenhouse conditions, and will discuss how these processes work.

2. The Atmosphere, Global Climate Change and Greenhouse Gases

The atmosphere of the Earth has evolved and changed over time and had reached a point of equilibrium. However, anthropogenic activities arising from the Industrial Revolution and subsequent development have changed Earth's atmospheric conditions. Since the industrial era began, a new phenomenon has been observed — that of global climate change (GCC). Many different sources are involved in the production of gases, with concern centering on the production of GHG in particular, as these gases are the ones implicated in the increasing rate of global warming on Earth. The main gases involved in this warming are methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2). Additional and important GHGs include water vapour, which has an effect on global climate change that can be used as a baseline with which to compare the remaining GHG. The production of these gases arises as a result of anthropogenic activities, mainly the combustion of fossil fuels (CO_2), extensive livestock and cattle farming (CH_4), and agriculture (N_2O) through denitrification or nitrification processes, and occasionally CO_2 depending on the type of fertilization employed.

As we can see, global climate change is a phenomenon caused by GHG that are emitted into the atmosphere. However, the main problem is not the emission of these gases, because these

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gases have actually been present in the Earth's atmosphere for thousands of years and they are the products of natural processes such as volcanic eruptions, plant and animal respiration and the microbial decomposition of organic matter. The contribution of human activity has resulted in the production of large amounts of these gases and their increased concentration in the atmosphere results in global warming. The most obvious effects of global warming are the continuous increase of global temperature and the changes in atmospheric conditions. All the elements of the environment are interrelated, and as a consequence, changes in one of them lead to changes in others. Sometimes these changes are small and imperceptible, while others can be very obvious. The rate of these changes is very important because if they are too rapid, then the ability of organisms to adapt to the new conditions might not be sufficient to ensure their survival as the natural process of adaptation takes thousands and thousands of years as a part of the evolutionary processes of life on Earth. The effects of these phenomena are the extinction of species and other serious negative effects on both the agriculture and fishing industries that are important economic activities the world over.

It is important to mention that the likely impacts of global warming could be different in different types of ecosystems because of the difference in climatic conditions in those ecosystems, but the effects on the abundance and distribution of biodiversity will be constant. All of these facts suggest that the natural conditions of the planet are being seriously affected by global climate change, global warming and GHG production, so we have a serious and worldwide environmental problem to address. However, there are many strategies, such as the use of alternative sources of energy, which could be implemented around the world to mitigate the damage being caused to the planet and to promote environmental awareness with favorable results in the future.

3. Experiments under *in vitro* conditions

We are interested in understanding which soil processes are involved in GHG production, and how they work, in several treatments or fertilizers. The production of CO_2, N_2O, and CH_4 when crops were grown under *in vitro* or greenhouse conditions was studied. These experiments were carried out using different types of soil (nitrogen depleted and/or alkaline-saline) and several crops were studied.

One of the experiments was conducted in order to investigate the evolution of nitrogen and its loss as a part of the nitrogen cycle. Different fertilizers or treatments were tested. These were ammonium sulphate [$(NH_4)_2SO_4$, 200 mg NH_4^+ kg^{-1}], wastewater sludge (200 mg NH_4^+ kg^{-1}), sterile wastewater sludge (200 mg NH_4^+ kg^{-1}) and a control (distilled H_2O). All of the treatments were added with KNO_3 at 100 mg N kg^{-1}, in two different soils (one agricultural and N depleted soil, and the second a saline-alkaline and N depleted soil) at 40% of water holding capacity (WHC) under *in vitro* conditions for 56 days. The variables were CO_2, N_2O, NH_4^+-N, NO_2^--N, and NO_3^--N, and were measured and assessed [4]. The soils used were specifics, i.e. the first soil was an agricultural soil which had mainly been cultivated with maize (> 25 years), low fertilization (< 50 kg N ha^{-1}) and was both C and N depleted (6.5 g organic C kg^{-1}, 0.2 g total N

Kjeldahl kg^{-1}, pH 7.8, electrolytic conductivity (EC) 1.0 dS m^{-1}, and the textural soil classification was loamy sand) from Otumba, in the State of Mexico (Mexico) (19° 42′ N, 98° 49′ W). The second soil was classified as an uncultivated soil (some grasses and small trees could be found) as a result of its former lake bed origin. It was found to be N depleted and alkaline-saline, pH 10.3, EC 12.4 dS m^{-1}, 49 g organic C kg^{-1}, and 0.6 total N Kjeldahl kg^{-1}, from Texcoco, State of Mexico (Mexico) (19° 30′ N, 98° 53′ W). The results showed that production of CO_2 from the Otumba soil was not affected by the addition of NH_4^+ or NO_3^-, i.e. both fertilizers produced a similar amount of CO_2, approximately 350 mg CO_2-C kg^{-1} dry soil. The sterilized sewage sludge increased the production of CO_2, > 1,000 mg CO_2-C kg^{-1} dry soil, i.e. over twice the production compared with that of the controls (soils treated with ammonium or nitrate). When wastewater sludge was added, the CO_2 production was ~ 3,100 mg CO_2-C kg^{-1} dry soil, a value twice that of sterilized sludge and eight times that of the controls. In the Texcoco soil, a similar contour was found in the CO_2-C dynamics. The control treatments present > 350 mg CO_2-C kg^{-1} dry soil. The soil treated with sterilized sludge showed a concentration of CO_2 that was > 1,100 mg CO_2-C kg^{-1} dry soil (2.1 times that of the controls) while the treatment with sewage sludge was ~ 2,100 mg CO_2-C kg^{-1} dry soil (over five times that of the controls). Different soils showed similar contours in C dynamics, and these results revealed that CO_2 production was not particularly different and that the processes involved were rather similar in the different soils investigated. Wastewater sludge is characterized by large amounts of organic matter (organic C) and it's suppose got a large amounts of organic matter easily decomposable. So, the sludge is easily and rapidly mineralized in both soils [4]. When sewage sludge was sterilized, the microbiota were destroyed and this might have affected the properties of the organic matter in the sludge [4]. It could be that the organic matter was more readily accessible for the soil microorganisms, so that the production of CO_2 should be higher in soils with sterilized sludge, but the results revealed that this is not necessarily true. The results also showed that the soil microorganisms and the sludge microorganisms could be exerting a synergistic action on the degradation of organic matter because the degradative action of the microorganisms of the soil or the sludge alone cannot improve the degradation of organic matter in the treated soil or in the sludge [4].

The ammonium dynamics showed that the initial concentrations of N were reduced after the first 3 days, and after that, a release of the mineral occurred from day 3 up to day 14. Later still, the concentration of ammonium decreased by up to < 14 mg N kg^{-1} dry soil for all the treatments in both the Otumba and Texcoco soils, and the ammonium concentration decreased by up to < 2 mg N kg^{-1} dry soil for all treatments, except for the soil treated with sterilized sludge, < 31 mg N kg^{-1} dry soil. The contour of the ammonium dynamics was similar in both the Otumba and Texcoco soils. Many abiotic and biotic processes might affect the concentration of NH_4^+ in soil, such as NH_4^+ fixation in the soil matrix, volatilisation of NH_3, and immobilization or oxidation of NH_4^+. Some soil processes were occurring at too low a level to be detectable, such as NH_4^+ fixation and the volatilisation of NH_3. The nitrate dynamics were similar in both soils. The concentration of NO_3^- was ~120 mg N kg^{-1} dry soil in the control treatment in both soils. The ammonium concentration was similar in both soils, > 200 mg N kg^{-1} dry soil, treatments with sludge reached > 255 mg N kg^{-1} dry soil and > 300 mg N kg^{-1} dry soil in the Texcoco and Otumba soils respectively, and soils treated with sterilized sludge increased the concentration to > 300 mg N kg^{-1} dry soil in the Texcoco soil, while in the Otumba soil it was > 325 mg N kg^{-1} dry soil. These results suggest that soil fertilized with wastewater sludge showed an increased NO_3^- concentration with a hypothetic mineralisation of ~ 60% at day 56 [4].

The production of N_2O was measured in both soils for seven days under C_2H_2 (10% v/v) conditions. The control treatment showed a N_2O production of < 0.02 mg N kg^{-1} with or without C_2H_2 in the Otumba soil. N_2O production increased when ammonium was added to the Otumba soil, 0.04 mg N kg^{-1}, but the addition of C_2H_2 reduced it to 0.01 mg N kg^{-1}. When the sterilized and non-sterilized sludge were added to the Otumba soil, N_2O production increased to 1 mg N kg^{-1} dry soil without C_2H_2 and 0.49 mg N kg^{-1} dry soil with C_2H_2. In the Texcoco soil, the control treatment was below 0.005 mg N kg^{-1} dry soil, but when C_2H_2 was added an increase to 0.09 mg N kg^{-1} dry soil was observed. Soil treated with ammonium increased the production of N_2O (0.04 mg N kg^{-1}), but under C_2H_2 conditions the concentration was low at 0.01 mg N kg^{-1} dry soil when compared with the control treatment. Soils treated with sterilized and non-sterilized sludge increased the production of N_2O (2.1 mg N kg^{-1} and 0.75 mg N kg^{-1}) compared with the control treatment, however, the addition of C_2H_2 increased the N_2O concentration of soil treated with sludge (2.1 mg N kg^{-1} dry soil) compared with soil treated with sterilized sludge (1.8 mg N kg^{-1}). It could be argued that the addition of easily decomposable organic matter into the soil will induce the denitrification process, where NO_3^- is reduced to N_2O and N_2 as final products. There are factors that could be important in controlling the production of N_2O in denitrification, such as oxygen, pH and the ratio of nitrate / available carbon [5]. There are additional parallel factors for NO that are less well understood [5]. It was evident that in a soil treatment of wastewater sludge or sterilized sludge, the N loss was increased. The microorganisms from the soil and the sludge were found to be working together in order to degrade the organic matter in the sludge. In addition, it could be suggested that more denitrifiers may be present in the Texcoco soil than in the Otumba soil, and a decrease of 50 mg NO_3^--N was counted at day 56, and the N_2O concentration was approximately double (plus 1 mg N_2O kg^{-1} dry soil) that of the Otumba soil on the day 7 [4]. When NO_3^- (an e^- acceptor) is present in excess compared to organic C (an e^- donor), the denitrifiers could be said to be "spendthrift" with respect to NO_3^- and in general produce N_2O as the major product. When the same NO_3^- is limited, the denitrifiers use it to its maximum potential as an e^- acceptor and reduce it all to N_2 (dinitrogen) [5]. Schimel and Holland (2005) explain that while the major producer of NO is nitrification, N_2O can also be produced in large quantities by nitrification or denitrification due to the fact that it is less reactive and can outflow from soils — even wet soils.

In our study, the microorganisms in the soil and sludge acted together synergistically in the reduction process, i.e. N_2O to N_2, the denitrification process, and in the Texcoco soil under C_2H_2 conditions, but the main contribution was from the soil microorganisms under the same conditions. In particular, the production of N_2 was almost 50% of the total gas evolved. When sterilized sludge was applied to the Texcoco soil, minimal N_2 was produced (~0.2 mg N_2O-N kg^{-1}). Furthermore, untreated soil showed an increase in N_2O when C_2H_2 was added (~0.07 mg N_2O-N kg^{-1}) and the N_2 produced was approximately 75% of the total gas evolved, under the conditions established. In the Otumba soil, the N_2O was produced by nitrification process (at

40% WHC), and showed in the soil treated with ammonium, sludge and sterilized sludge at 0.04, 1.0, and 1.0 mg N_2O-N kg^{-1} respectively, when compared with untreated soil. N_2O production was low when C_2H_2 was added to the Otumba soil, and no N_2 was produced under these conditions. It can be established that when organic matter with a high N content is added to soil, it significantly increases N_2O production compared to untreated soil or soil fertilized with ammonium [$(NH_4)_2SO_4$] in both soils (an ordinary soil and an alkaline-saline soil). The major source of production of N_2O was found in all treated soils to be as a result of the nitrification process, and the production of N_2 was not recorded in the Otumba soil in this experiment. In the Texcoco soil, the major source of production of N_2O was as a result of the denitrification process by microorganisms in the soil, and the production of N_2 was approximately 50% of the total gas evolved (1.1 mg N_2O-N kg^{-1}).

4. Experiments under greenhouse conditions

4.1. First experiment under greenhouse conditions

Subsequent studies were established in order to better understand plant growth and the production of GHG (CO_2 and N_2O) when a regular *Bacillus subtilis* strain was inoculated on the surface of the sunflower (*Helianthus annuus* L.) cultivar seeds under greenhouse conditions. The *B. subtilis* strain was characterized as PGPR, i.e. showing antagonistic activity against *Fusarium oxysporum* and *Rhizoctonia solani* AG1, phosphate solubilizing activity, 1-aminocyclopropane-1-carboxilate deaminase, and indole-3-acetic acid production. The strain was found as regular PGPR, for more details see [6].

The soil was collected from Alcholoya (Acatlán, in the State of Hidalgo, Mexico). This soil is an agricultural soil with a pH of 6.5, electrolytic conductivity (EC) 0.7 dS m^{-1}, 846 g kg^{-1}, organic C content was 11.1 g C kg^{-1} soil, and total N content 1.0 g N kg^{-1} soil. The soil was sampled from three different plots (400 m^2), ~ 800 kg was obtained and each plot was pooled separately and passed through a 5 mm sieve. Thirty-six sub-samples of 6.5 kg of soil from each plot were placed in cylindrical pots (Ø = 16 cm, 50 cm) with 7 cm of gravel in the bottom. Four treatments were applied, with nine pots for each of the three soil sites sampled ($n = 27$). The first treatment was unfertilized and uncultivated soil (used as the CONTROL treatment), the second treatment was unfertilized soil cultivated with sunflowers (SUNFLOWER treatment), the third treatment was soil cultivated with sunflowers and fertilized with 0.5 g urea (75 kg N ha^{-1}, UREA treatment), and the fourth treatment was soil cultivated with sunflowers (seeds were dressed with *B. subtilis*) and fertilized with 0.5 g urea (BS treatment). All treatments were irrigated with tap water, with an additional input of 19 kg mineral-N ha^{-1} as NO_2^--N and NO_3^--N. In addition, twelve days after the emergence of the plantlets, they were fertilized with another 0.5 g of urea (the UREA and BS treatments), giving a total amount of 150 kg N ha^{-1}; and three weeks after sowing the plantlets were drenched with 4 mL of a bacterial suspension (at the same concentration as described above) adjacent to the plantlet roots at a depth of 3 cm. From the beginning of the experiments and approximately every two days for the following 30 days, the pots were

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closed air-tight and their atmosphere was analysed for CO_2 and N_2O at times of 0, 3, 15 and 30 mins. The experiment was repeated twice [6].

The daily CO_2 production rate for some treatments was large at the beginning of the experiments (data not shown). The daily CO_2 production rate showed a drop, remaining < 5 mg C kg^{-1} day^{-1} on day 2 and after that it remained at < 8 mg C kg^{-1} day^{-1} for all treatments up to end of the experiments. The mean CO_2 production rate was not significantly different between treatments. The daily N_2O production rate remained ≤ 0.75 μg N kg^{-1} day^{-1} for the SUNFLOWER and CONTROL treatments. Meanwhile, in the BS and UREA treatments, the daily N_2O production rate remained ≤ 2.1 μg N kg^{-1} day^{-1} with a maximum score of production in the first 14 days. The mean of the N_2O production rate of BS treatment was significantly high when compared with the SUNFLOWER and CONTROL treatments. Cultivating soil with sunflowers (SUNFLOWER treatment) did not affect the production of CO_2 compared with the CONTROL treatment (uncultivated soil). It is well known that cultivated soil frequently increases the production of CO_2, possibly due to the activities of the microorganisms degrading the easily decomposable organic matter such as the dying roots and root exudates in the rhizosphere, thereby increasing the production of CO_2 from the soil. Soils cultivated with sunflowers and fertilized with urea did not affect the production of CO_2. Applying urea to soil commonly has no effect on the production of CO_2 from soils. However, the production of CO_2 might be stimulated when urea is applied to N depleted soil as reported in Phillips and Podrebarac (2009), where the CO_2 production was tripled when 112 kg urea-N kg^{-1} was applied to an arable soil. Increases in CO_2 production with several doses of urea-N application indicate that agronomic-scale N inputs might stimulate microbial carbon cycling in arable soils [7]. The inoculation of *B. subtilis* on sunflower roots in soils fertilized with urea did not show any effect on the production of CO_2 compared with the UREA treatment. The application of UREA to the soil resulted in more than a doubling of the mean N_2O production rate when compared with the CONTROL treatment. The production of N_2O and NO in the soil is the result of factors such as ion concentrations and soil conditions under chemical disequilibrium, i.e. the oxidative process of NH_4^+ to NO_3^- under aerobic conditions, nitrification, and a reductive process of NO_3^- to N_2 under anaerobic conditions, and denitrification or the nitrifier denitrification [5, 8, 9]. According to the IPCC, N_2O is the main gas produced and released to the atmosphere by soil microorganisms [9]. So, when urea is applied into the soil, hydrolysis of the urea is immediately started, releasing NH_4^+ into the soil, which is rapidly transformed to NO_3^- with the simultaneous production of N_2O by the same process. A high concentration of NO_3^- in soil favours the production of N_2O due to the presence of anaerobic micro-sites in highly compacted soil.

A principal component analysis (PCA) was undertaken to investigate several plant biometric parameters and soil properties in the production of N_2O and CO_2. The analysis was carried out to include all variables. The PCA revealed that BS treatment has an effect on the shoots of plants, i.e. the shoot length, dry weight of shoot, and the fresh weight of shoot. UREA treatment has an effect on roots, i.e. the dry weight of roots, length of roots, and fresh weight of roots, and a minimal effect on seed weight. Otherwise, the soil properties PCA showed that both the BS and UREA treatments had an effect on NO_3^- and EC at depths of 0-15 cm and 15-30 cm. The

BS treatment also has effect on the production of N_2O and the UREA treatment has an effect on NH_4^+ at a depth 0-15 cm and a slight effect on CO_2 production. These results were found to correspond with a production rate of N_2O that was ≤ 2.1 μg N kg^{-1} day^{-1} with a maximum production in the first 14 days of more than that in the UREA treatment (< 1.5 μg N kg^{-1} day^{-1}). Additionally, the PCA showed that *B. subtilis*, as a regular strain, had a marked effect on the production of N_2O but not on CO_2 production. This strain might be involved in nitrifier denitrification (or aerobic nitrification-denitrification) as was reported by Kim et al. [10] and Yang et al. [11]. Both research groups demonstrated that the *Bacillus* genus is involved in nitrification and denitrification, namely *B. subtilis*, *B. cereus* and *B. licheniformis*, where *B. subtilis* is involved in the nitrification process and *B. licheniformis* is involved in denitrification or aerobic denitrification [10]. Yang et al. reported that the strain of *Bacillus subtilis* A1 is an aerobic heterotrophic nitrifying–denitrifying bacterium, which is able to convert NH_4^+ to N_2 under fully aerobic conditions, while growing either autotrophically or heterotrophically [11]. In our experiment, the environmental condition of the soil was aerobic throughout the experiment, so it could be hypothesized that the *B. subtilis* strain could be involved in the nitrification process or the nitrifier denitrification process (or aerobic denitrification).

4.2. Second experiment under greenhouse conditions

The second experiment was carried out using wastewater sludge as an organic fertilizer. It tested the effect of the sludge or urea on sunflower growth, and the effect of some soil properties on the production of CO_2 and N_2O. The plant characteristics were also evaluated. Wastewater sludge or sewage sludge is generated during wastewater treatment and is an unavoidable by-product. However, the sludge can be seen as an invaluable by-product when it is applied to the soil as stabilized sludge. Also, this waste management is the most economical form of disposal employed to reduce the large amount of sewage sludge. In addition, wastewater sludge is organic matter rich in minerals and is an outstanding source of C and N, *inter alia*. Applying sludge to the soil offers the opportunity of recycling nutrients for use by plants, while at the same time returning C as organic matter to the soil in order to improve agriculture processes.

This experiment was carried out in the same way as the previous experiment (described above). The wastewater sludge was collected from the Reciclagua treatment plant, S.A. de C.V., where wastewater from various industries (including the food industry) and households is treated. The properties of the sewage sludge were pH 8.1, EC 7.9 dS m^{-1}, water content 847 g kg^{-1}, organic C content 288 g kg^{-1}, total N content 41.8 g kg^{-1}, NH_4^+ 13 g N kg^{-1}, NO_2^- 8.3 mg N kg^{-1}, and NO_3^- 122 mg N kg^{-1}. For more details of these, see [12]. Four treatments were established in cylindrical pots, comprising nine soil samples from three sampled plots ($n = 27$). The treatments were: i) unfertilized and unsown soil (CONTROL treatment), ii) unfertilized soil cultivated with sunflowers (SUNFLOWER treatment), iii) cultivated soil fertilized with 0.5 g urea (0.5 g urea × 2 applications, equivalent to 150 kg N ha^{-1}, UREA treatment), and iv) soil cultivated and fertilized with 30 g sludge (SLUDGE treatment). The sludge was added so as to be equivalent to 150 kg N ha^{-1}, assuming that sludge mineralisation was 40% mineral N during the crop cycle. Tap water supplied a total amount of 19 kg mineral-N ha^{-1} through irrigation in all treatments and throughout the experiment. In order to measure the production of gases, the pots were closed airtight approximately every two days for the first 30 days, and their atmospheres were analysed for CO_2 and N_2O at sequential times of 0, 3, 15 and 30 mins. The experiment was replicated twice [12].

The results showed that the CONTROL, SUNFLOWER and UREA treatments were not significantly different with respect to the production rate of CO_2 (1.59, 2.03 and 2.6 mg C kg^{-1} day^{-1}, respectively) (Table 1). The CO_2 production rate of sunflowers cultivated in soil fertilized with sewage sludge (SLUDGE treatment) was 2.96 mg C kg^{-1} day^{-1} and was significantly different compared with the CONTROL treatment. In other words, soil cultivated and fertilized with sewage sludge was equivalent to both the soil cultivated and fertilized with urea, and the unfertilized soil. It should be taken in account that several factors affect CO_2 production in the soil, such as rhizosphere respiration and soil microbial respiration, soil moisture, soil temperature, substrate quantity and quality, vegetation type, and land use and management regimes [13].

Treatment	CO_2 mg C kg^{-1} day^{-1}	N_2O μg N kg^{-1} day^{-1}
CONTROL	1.59 B	0.18 B
SUNFLOWER	2.03 AB	0.27 B
UREA	2.60 AB	0.66 B
SLUDGE	2.96 A	2.50 A
Least significant difference	1.14	0.74
Standard error of estimate ($P < 0.05$)	0.41	0.26

Values with the same letter show no significant difference between treatments ($P < 0.05$).

Table 1. The production rates of CO_2 and N_2O from soil cultivated with *H. annuus* under greenhouse conditions.

On the other hand, the rate of production N_2O was significantly different in cultivated soil fertilized with sludge, 2.5 μg N kg^{-1} day^{-1} compared with the remaining treatments, 0.7, 0.3, and 0.2 μg N kg^{-1} day^{-1} for the UREA, SUNFLOWER, and CONTROL treatments respectively. As previously discussed, wastewater sludge is an organic matter which is rich in easily decomposable material. In addition, it has been demonstrated that microorganisms from the sludge plus those from the soil work together synergistically to accelerate the decomposition of organic matter [6]. The high levels of microbial activity stimulated by the addition of material with high C and N contents could increase the production of both CO_2 and N_2O. According to Kool et al. [9], the loss of N in our experiment might primarily be as a result of the nitrifier nitrification process (from ammonium oxidation) followed by the nitrifier denitrification process. As a result, the wastewater sludge had a NH_4^+ concentration of 13 g N kg^{-1}, an amount of ammonium which might not be oxidized to N_2O so rapidly. The chemical composition of organic or mineral fertilizers — or even residues applied to the soil — is an important factor in regulating the magnitude of N_2O production.

A PCA was performed on all relevant properties of the soil. The first principal component explained about 22% of the observed variation, while the second accounted for 17% of the observed variation. On the related scatter plot, the UREA treatment lies in upper right quadrant and the SLUDGE treatment is in the upper left quadrant. The SUNFLOWER and CONTROL treatments were found in lower left and right quadrants respectively (Figure 1).

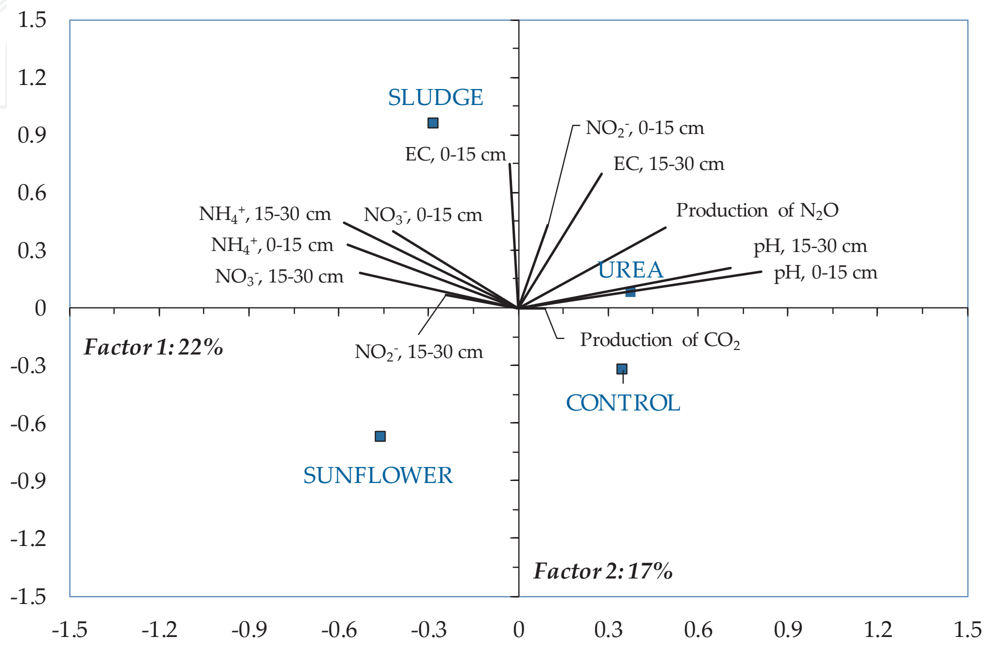

Figure 1. Results of a principal component analysis (PCA) performed on soil properties under greenhouse conditions ($n = 27$).

The UREA treatment seems to have an effect on the pH of soil at both depths (0–15 and 15–30 cm) and also affects the production of CO_2 and N_2O. The PCA also revealed that the SLUDGE treatment affected EC at a depth of 0–15 cm and had a slight effect on both ammonium and nitrate concentrations at both depths.

4.3. Third experiment under greenhouse conditions

In this study, the effect of urea, wastewater sludge and vermicomposting on the production of CO_2 and N_2O was investigated. The Otumba soil (State of Mexico, Mexico), which was characterized as a sandy loam with pH 7.6, EC 1.15 dS m^{-1}, and an organic C content of 7.2 g C kg^{-1}, was used in this study. Wastewater sludge was again collected from Reciclagua S.A. de C.V. (as described above). The vermicompost was prepared with wastewater sludge from Reciclagua and *Eisenia fetida*. The vermicompost was obtained from a mixture of sludge (1,800 g) and manure (800 g) with 70% water content, and was added to 40 individuals of *E. fetida* with the mixture being conditioned over three months. The properties of the vermicompost were pH 7.9, EC 11 dS m^{-1}, organic C content 163 g kg^{-1}, and a total N content of 2 g kg^{-1}. As well, the vermicompost presented < 3 CFU g^{-1} *Salmonella* sp., no *Shigella* sp., and no helminth ova.

Forty-five sub-samples of 3.25 kg soil were prepared, i.e. three soil samples, three replicates, and five treatments were established. The treatments were: i) soil fertilized with 0.07 g urea kg^{-1}, (UREA treatment), ii) soil fertilized with 21.2 g sewage sludge kg^{-1} (H-SLUDGE treatment), iii) soil fertilized with 12.8 g sewage sludge kg^{-1} (L-SLUDGE treatment), iv) soil fertilized with 81.5 g vermicompost kg^{-1} (VERMI treatment), and v) unfertilized soil (CONTROL treatment). The amount of urea was 80 kg N ha^{-1} for the UREA, H-SLUDGE, and VERMI treatments, while the L-SLUDGE treatment was 48 kg N ha^{-1}. The production of CO_2 and N_2O was analysed every two days at 0, 3, 15 and 30 mins, until day 97. The cultivar of common bean used was Negro-8025 (from Universidad Autónoma Chapingo, Texcoco, State of Mexico). Tap water was used for irrigation at a rate of 500 mL every seven days. The experiments were triplicated and ran for 117 days in total.

A large amount of CO_2 was recorded at the end the experiment (from day 45 until day 82). The UREA treatment had no significant effect on CO_2 production when compared with the CONTROL treatment (0.043 mg C kg^{-1} dry soil). Wastewater sludge was found to increase the mean production of CO_2 in the soil at 0.064 mg C kg^{-1} compared with the untreated soil, and the VERMI treatment showed the largest mean CO_2 production at 0.1 mg C kg^{-1}. Urea had no significant effect on CO_2 production when compared with the CONTROL treatment. Occasionally, urea might stimulate the production of CO_2 in N depleted soils. Wastewater sludge increased CO_2 production through the mineralization of organic C and the increasing of microbial activity in the rhizosphere. Similarly, vermicomposting stimulated plant growth and increased root exudates and microbial activity in the rhizosphere. The high levels of CO_2 produced towards the end of the experiments could be related to rapid root growth and the beginning of root decomposition. For example, common bean plants at 49 days after sowing (flowering and nodule senescence begins) showed a decrease in the number of nodules, and their nodule cell walls slowly became thinner and degraded [14] thereby improving the environment for the growth of microorganisms and increasing the production of CO_2 towards the end of the experiments.

The mean production of N_2O was -0.004 μg N kg^{-1} in the CONTROL treatment. Soil fertilized with urea increased the mean production of N_2O to 0.015 μg N kg^{-1}. Wastewater sludge increased the mean production to 0.11 μg N kg^{-1} and 0.58 μg N kg^{-1} in the L-SLUDGE and H-SLUDGE treatments respectively, and the vermicompost mean was 0.32 μg N kg^{-1} dry soil. N_2O production in order of effect size of treatment was: wastewater sludge (H-SLUDGE) > vermicomposting > wastewater sludge (L-SLUDGE) > urea > unfertilized soil. Nitrifier nitrification and nitrifier denitrification were presumably the processes that contributed the most to the production of N_2O under aerobic conditions.

4.4. Fourth experiment under greenhouse conditions

Juárez-Rodríguez et al. applied the sludge derived from anaerobically digested cow manure in the production of biogas (methane-air), to maize (*Zea mays* L.) cultivated in a nutrient-low, alkaline-saline soil with EC 9.4 dS m^{-1} and pH of 9.3. The results showed that the CO_2

production increased 3.5-fold in the soil cultivated with maize and sludge, and increased 3.1-fold after the sludge was added to the soil. The production of CO_2 from soil cultivated with maize showed a 1.6-fold increase compared with the uncultivated and unfertilized soil, 1.5 mg C kg^{-1} day^{-1}. N_2O production was -0.0004 μg N kg^{-1} soil day^{-1} in unfertilized soil, and in soil cultivated with maize was 0.3 μg N kg^{-1} day^{-1}. Soil treated with sludge increased the production of N_2O up to 4.6 μg N kg^{-1} soil day^{-1}. Nevertheless, it was found that cultivated soil produced 2.4 μg N kg^{-1} soil day^{-1}, reducing N_2O production. It was also found that applying the anaerobically digested cow manure stimulated the growth of maize cultivated in an alkaline and saline soil, and the production of CO_2 and N_2O was increased.

4.5. Fifth experiment under greenhouse conditions

In this study, the main aim was to investigate how maize fertilized with wastewater at 120 kg N ha^{-1} affected crop growth, soil properties and the production of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) compared with plants fertilized with urea [16].

The soil was collected from the Mezquital Valley, located near Pachuca in the State of Hidalgo (Mexico). The irrigation water used was slightly alkaline with a pH of 8.4. The experiment was carried out under greenhouse conditions. Soil collected from three sub sites was placed into cylindrical pots. Five treatments were established in order to study the effect of wastewater and urea on the cultivation of maize (*Zea mays* L.). The treatments were: a) SMWW, maize plant plus wastewater; b) SMUREA, maize plant plus urea as fertilizer; c) SUREA, uncultivated soil and urea as fertilizer; d) SWW, uncultivated soil plus wastewater; and e) SCONTROL treatment, soil plus tap water. Soils from the SMWW and SWW treatments were irrigated with 1000 mL of wastewater every 7 days from the first day onwards, making a total of 13 times overall. This means that a total amount of mineral N equivalent to 120 kg N ha^{-1} was added to each maize plant, i.e. the recommended amount of N fertilizer for maize.

The concentration of NH_4^+ was larger in the soil treated with urea and wastewater than in the untreated soil, as the urea was hydrolysed and the wastewater contained high concentrations of NH_4^+. The addition of wastewater to the soil doubled the production of CO_2 and approximately 0.2 g C was produced from the soil due to the decomposition of the wastewater after 70 days. In other words, 34% wastewater C was mineralized. However, urea may only occasionally stimulate CO_2 production when a soil is N depleted. Plants take CO_2 from the atmosphere, but mineralization of root exudates increases the production of CO_2. The production of CO_2 increased towards the end of the period of maize growth. This indicated that the phenological stage of the plants affected CO_2 production. The growth of maize plants was similar under the SMWW (wastewater) and the SMUREA (urea) treatments, even when the release of nutrients was delayed by mineralisation from the organic matter in the wastewater. When wastewater was applied to the soil, the mean production rate of CO_2 increased significantly at 2.4-fold, 1.7 μg C kg^{-1} h^{-1}, compared with the SCONTROL treatment at 0.7 μg C kg^{-1} h^{-1} (Table 2). Meanwhile, cultivating maize increased CO_2 production 3.2-fold, 5.6 μg C kg^{-1} h $^{-1}$. The SWW, SMWW or SUREA treatments did not show a significant difference in the production of N_2O compared with the SCONTROL (1.5×10^{-3} μg N kg^{-1} h^{-1}). The addition of urea did not affect the CH_4 oxidation rate (0.1×10^{-3} μg C kg^{-1} h^{-1}), nor did the SMUREA treatment (cultivated soil fertilized with urea), but the addition of wastewater to the soil significantly increased CH_4 production to 128.4×10^{-3} µg C kg^{-1} h^{-1}. Soil irrigated with wastewater increased the global warming potential (GWP) up to 2.5-fold compared with the SUREA treatment (soil plus urea), whereas cultivated soil increased the GWP 1.4-fold. Crops irrigated with wastewater might limit the use of N fertilizer and water from aquifers. Nevertheless, the amount of fertilizer applied must be limited due to nitrate (NO_3^-) leaching and the production of CO_2, N_2O and CH_4 – that they could be produced in significant amounts –, and at the same time the salt content of the soil will accumulate, limiting the growth of the crop.

Soils can be either a net sink or a net source of CH_4, depending on several factors such as the moisture level, N level, and the nature of the ecosystem in question. Methane is used up by methanotrophic microorganisms, which are ubiquitous in several soils, and is produced by methanogenic microorganisms in the soil under anaerobic conditions. Agricultural systems are not normally large sources or sinks of CH_4. Only under certain conditions are they sources of CH_4 — after application of manure or other organic materials, or moderate to high levels of irrigation. Our results showed that soil irrigated with wastewater — with or without maize — increased CH_4 production significantly (SMWW and SWW treatments) particularly after irrigation, due to temporary anaerobic conditions.

Treatment	CO_2 (µg C kg^{-1} h^{-1})	N_2O (µg C kg^{-1} h^{-1})	CH_4 (µg C kg^{-1} h^{-1})	GWP [a] (g C kg^{-1} soil)
SMWW	5.6 A[b]	2.8×10^{-3} A	163.6×10^{-3} A	1.97
SMUREA	4.95 A	4.5×10^{-3} A	8.4×10^{-3} C	1.44
SUREA	0.9 C	3.3×10^{-3} A	0.1×10^{-3} C	0.36
SWW	1.7 B	2.5×10^{-3} A	128.4×10^{-3} B	0.90
SCONTROL	0.7 C	1.5×10^{-3} A	1.5×10^{-3} C	0.26

[a] The global warming potential (GWP) of the gases produced was calculated considering CO_2 production equivalent to 310 for N_2O, 21 for CH_4 and 1 for CO_2 (IPCC, 2007) over a 90-day period, minus the C that was stored in the roots per kg soil.

[b] Values with the same letter show no significant difference between treatments ($P < 0.05$).

Table 2. Production of greenhouse gases, CO_2, CH_4 (µg C kg^{-1} soil h^{-1}), and N_2O (µg N kg^{-1} soil h^{-1}) from five treatments: a) soil + plant + wastewater (SMWW), b) soil + plant + urea (SMUREA), c) soil + urea (SUREA), d) soil + wastewater (SWW), and e) soil + water (SCONTROL).

Fertilizing maize with urea or wastewater had a similar effect on plant growth, so wastewater might be useable as a crop fertilizer. The treatments with urea or wastewater had no effect on the pH of soil in this experiment due to the fact that the soil is a vertisol, characterized by a clay type 2:1, with a large capacity for the exchange of protons and consequently, a high buffering capacity. The addition of wastewater increased the production of both CO_2 and CH_4 compared with the soil treated with urea, but did not increase the production of N_2O. The irrigation of crops with wastewater might in the long term be a far more environmentally friendly approach to that of using water from aquifers that take long periods of time to fill, as long as the amount of wastewater applied is restricted to the amount required by the cultivated crop due to possible substantial losses of mineral N through several process such as the production of CO_2, CH_4 and N_2O, and the fact that soil salinization could increase rapidly.

5. Conclusions

The organic fertilizers or treatments (vermicompost, wastewater sludge, anaerobically digested cow manure, and wastewater) might increase the production of greenhouse gases, as do several abiotic and biotic factors involved in microbial activity within the soil. Sludge as a soil fertilizer offers the opportunity for the recycling of plants nutrients and the recovery of C as organic matter and its use in soil to improve agriculture. The high levels of microbial activity stimulated by the addition of this high C and N content material could increase both CO_2 and N_2O production. It should be taken in account that several factors are involved in the production of gases in the soil such as rhizosphere respiration, vegetation type, and soil microbial respiration, as well as abiotic factors such as soil moisture, soil temperature, substrate quantity and quality, and land use and management regimes.

Environmental and economic implications must be considered in order to make well-informed decisions on the management of soil treatments, i.e. how many, how often and what kind of organic fertilizer should be used in order to improve crop production and simultaneously limit soil deterioration and greenhouse gases production.

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