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Chapter 1

General introduction

Modern agriculture is dependent of mineral fertilizers and it is expected that this will increase in the next decades. World fertilizer nutrient (N+P₂O₅+K₂O) consumption was estimated to be around 187 million tons in 2016 (FAO, 2017). In order to reduce the abundant use of mineral fertilizers the recycling of organic residues and the optimization of the use of nutrients in agriculture are widely used strategies. Organic residues are produced in huge amounts and in some extent have been considered contaminants. However, the application of organic residues as fertilizer is one of the best options to decrease this problem. Organic residues can be an important source of nutrients for crops, especially in regions nearby the production site, and these residues can replace a significant portion of the inorganic fertilizers input (Ussiri et al., 2009; Christofolletti et al., 2013; Trivelin et al., 2013). Furthermore, application of organic residues has been proposed as a useful option to improve soil structure and protection by reducing erosion and runoff (Rossetto et al., 2010; Boulal et al., 2011; Bhattacharyya et al., 2013; Jemai et al., 2013; Brouder and Gomez-Macpherson, 2014; Carvalho et al., 2017; Menandro et al., 2017). However, the inadequate and indiscriminate discharge of residues in the environment may cause an unwanted disturbance of the soil system. If residues are applied beyond the soil retention capacity or above the plant nutrient requirements soil, water and atmosphere contamination may occur (Carmo et al., 2013; Di et al., 2014; Navarrete et al., 2015a; Pitombo et al., 2015; Tao et al., 2015; Castro et al., 2017). Besides, the application of organic residues in the soil may also affect seriously the soil microbial community and consequently the process carried out by the soil biota including the production of greenhouse gases (GHG), *i.e.* CO₂, CH₄ and N₂O as it has been observed after vinasse and sewage sludge applications in soil as fertilizer (Carmo et al., 2013; Pitombo et al., 2015; Tao et al., 2015; Soares et al., 2016; Suleiman et al., 2016).

Microbial communities can change abruptly in response to perturbations and may recover quickly to its original state. Understanding of how organic residues in combination with mineral fertilizer and seasonal climatic variations affect the diversity, composition and dynamics of the resident soil microbes is required to reduce negative side effects of its application in agriculture. Only by using time series approaches the stability and dynamics of microbial communities' response to perturbations can be assessed properly. Thus, the main objectives of the study described in this thesis are to assess the impact of bioenergy organic residue amendments, *i.e.* vinasse and sugarcane straw, on the structure and functioning of the soil microbial community and to determine the link with the nitrous oxide (N₂O) production and emission, which is the most important GHG emitted from sugarcane soils (Cerri et al., 2009), after the application of these residues. The information from this study may help to develop and implement sustainable agricultural cropping systems in which recycling of residues is linked with adequate nutrient management without side effects of GHG's emissions and/or nutrient runoff and leaching.

1. *Vinasse*

Brazil is the world's largest producer of sugarcane, and the second largest producer of ethanol, with about 685 million tons of sugarcane produced in 2016/2017 on an area of 9 million hectares (CONAB, 2017). São Paulo state has the largest area of sugarcane, approximately 52% of the total area of sugarcane in Brazil. Moreover, 53% of the total Brazilian sugarcane production is destined for the production of ethanol (CONAB, 2017). Up to date, ethanol from sugarcane is considered one of the most economical and sustainable biofuels in the world so far (Goldemberg et al., 2008) and one of the best options to replace fossil fuels (Lisboa et al., 2011). Studies conducted by Macedo et al. (2008) and Seabra et al. (2011) indicated that ethanol emits about 80% less GHG's than gasoline. However, some management practices may counter this benefit, for example, the recycling of the residues generated during the ethanol production in the sugarcane fields as organic fertilizer and the application of inorganic nitrogen (N) fertilizer (Galdos et al., 2010; De Figueiredo and La Scala Jr, 2011; Carmo et al., 2013; Pitombo et al., 2015; Siqueira Neto et al., 2016). Depending on the management practices, the N₂O emitted from organic and inorganic fertilization during the sugarcane crop season can increase the total amount of GHG emitted to the atmosphere from the production and use of ethanol to a level similar to that of the use of fossil fuel (Crutzen et al., 2008; Lisboa et al., 2011; Carmo et al., 2013).

Vinasse is a major residue generated during sugarcane fermentation to ethanol (Figure 1). For each liter of ethanol produced, 10 to 15 liters of vinasse are generated. It was estimated in 2016/2017, that Brazil produced up to 360 billion liters of vinasse per year (27.5 billion liters of ethanol) (CONAB, 2017). Vinasse is a dark-brown wastewater with high organic content (biochemical oxygen demand of 2-20.8 mg L⁻¹ and chemical oxygen demand of 2-49.5 mg L⁻¹), rich in potassium (2056 mg L⁻¹), and nitrogen (357 mg N L⁻¹) (Elia-Neto and Nakahodo, 1995; Macedo et al., 2008; Christofolletti et al., 2013; Fuess and Garcia, 2014). The chemical composition of sugarcane vinasse is quite variable and varies with sugarcane variety, stage of plant development, soil type and distillation process (Christofolletti et al., 2013; Mutton et al., 2014) (Figure 1). Thus, effluents (vinasse) from the distillation of molasses, sugarcane juice or the combination of both are different, depending on whether the industry is producing ethanol or sugar in a certain period of the year (Christofolletti et al., 2013; Fuess and Garcia, 2014). Higher sugar production rates increase the volumes of molasses, a residue that is obtained after evaporation and crystallization and subsequently directed to the production of ethanol (Figure 1), providing vinasse with high levels of organic and inorganic compounds. In contrast, the direct use of sugarcane juice to fermenters provides a more diluted vinasse in terms of organic and inorganic compounds.

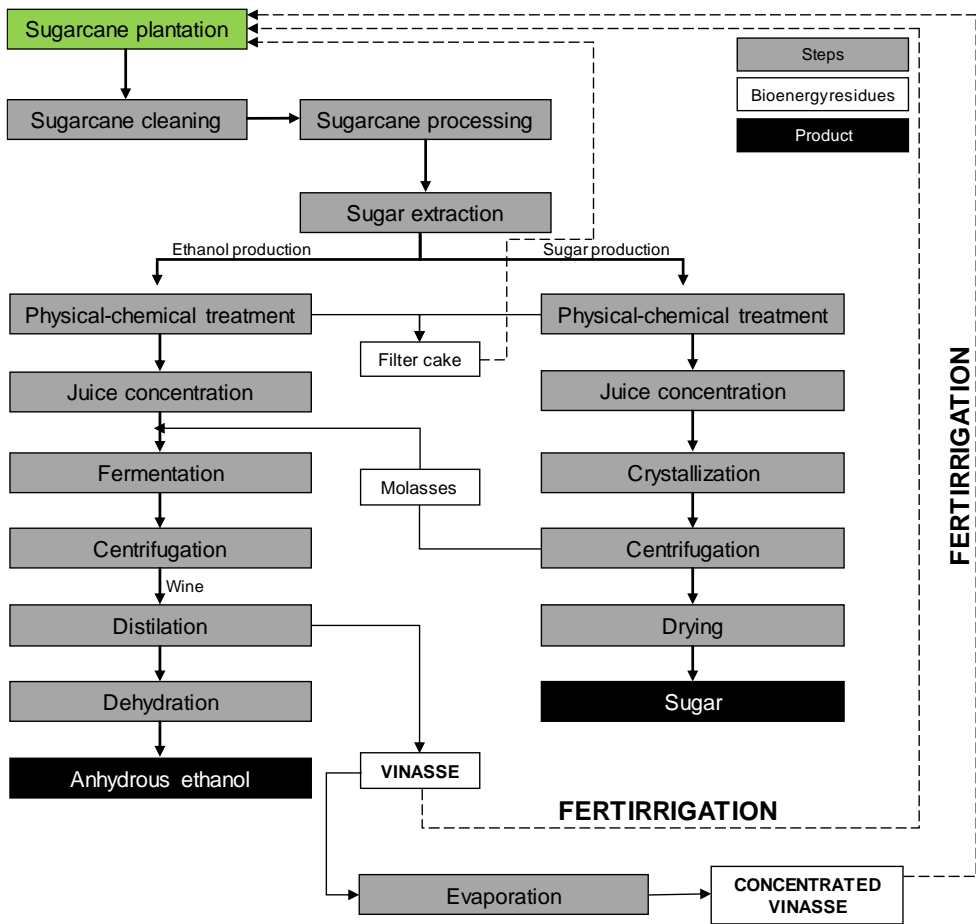


Figure 1 | Simplified flowchart of Brazilian sugarcane-based biorefineries for the production of ethanol and sugar and the associated production of residues; adapted from Fues et al. (2017).

Because of its chemical characteristics, especially the potassium concentration, vinasse is often directly applied on sugarcane fields as liquid organic fertilizer, which process is called fertirrigation (i.e., the utilization as a liquid fertilizer for plants) (Silva et al., 2014). Although there are different methods to recycle vinasse, including the use as fodder (Christofolletti et al., 2013), incineration for production of energy (Akram et al., 2015) and fermentation (Moraes et al., 2015), fertirrigation is the number one management method of vinasse recycling in Brazil. Due to the high amount of potassium as mentioned before, its effectiveness in terms of potassium fertilization is equivalent to that of an inorganic fertilizer. By law the concentration of potassium in both soil and vinasse must be taken into account for proper application of vinasse in sugarcane fields (Uyeda et al., 2013; CETESB, 2014), as high levels of vinasse may cause soil and groundwater contamination. However, vinasse cannot always be used in fertirrigation due to the

huge volume and high costs of transport to the field. Concentration of vinasse by evaporation, therefore, is an option to reduce the volume without loss of nutrients and so to reduce the transportation costs (Christofoletti et al., 2013). This procedure increased largely in recent years. Concentrated vinasse is applied in the plant row similarly to the application of inorganic fertilizer allowing higher amounts of nutrients close to the plants. However, there is little information about the efficiency of concentrated vinasse as fertilizer and information on its environmental impacts is scarce.

Despite its benefits, ethanol from sugarcane has been highly criticized for its negative environmental effects (Fuess et al., 2017; Rodrigues Reis and Hu, 2017). One of the main points of criticism concerned the use of vinasse. Vinasse has been shown to have negative effects on soil, groundwater and crops on the long term (Christofoletti et al., 2013). Vinasse can cause soil salinization, as the continuous application of this residue leads to the accumulation of salts in the soils. The acid characteristic of the vinasse (pH 3.0–4.7) can also cause acidification of water resources (Fuess et al., 2017; Rodrigues Reis and Hu, 2017). The input of organic carbon and organic N from vinasse, may lead to the reduction of the oxygen present in soil and groundwater directly effecting the microbial activity, and consequently changing soil processes, for example favoring denitrification and so N₂O production (Carmo et al., 2013). While for many agriculture and industrial residues (e.g., municipal wastewater, swine manure), a vast literature about the impact of residues on soil physical, chemical and biological constitution, including the resident soil microbial community, is available, for vinasse this information is limited. In addition, the process of ethanol production from sugarcane does not occur under sterile conditions and, so, the contamination of soil and water by microbes inhabiting the vinasse complex may also occur (Costa et al., 2015a; Brexó and Sant'Ana, 2017). In general, the main contaminants during ethanol production include *Acetobacter*, *Bacillus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Staphylococcus*, *Streptococcus* and *Weissella* (Costa et al., 2015a; Brexó and Sant'Ana, 2017). Costa et al. (2015a) found that after fermentation of the wine stage where vinasse is produced, *Lactobacillus* dominated the microbial community of contaminants. To our knowledge, up to date, there is no study on the fate of microbial contaminants in the vinasse residue, consequently no study has been published on the potential invasion of these microbes in soils receiving the vinasse.

2. Microbial community responses to disturbances

The microbial community composition of soils is influenced by physical, chemical, and biological factors, and by management and environmental disturbances. These disturbances include tillage (Sengupta and Dick, 2015), cover cropping (Navarrete et al., 2015a), crop rotation (Soman et al., 2016), fertilization (Su et al., 2015; Cassman et al., 2016), and organic amendments (Navarrete et al.,

2015a; Suleiman et al., 2016; Lupatini et al., 2017). Furthermore, also soil type, (Ulrich and Becker, 2006; Wakelin et al., 2008; Lupatini et al., 2013a; Mendes et al., 2015a; Mendes et al., 2015b), pH and other chemical factors (Lauber et al., 2008; Kuramae et al., 2011; Kuramae et al., 2012; Navarrete et al., 2015b; Ying et al., 2017), moisture (Stark and Firestone, 1995; Valverde et al., 2014), and temperature (Lipson, 2007; Prevost-Boure et al., 2011), as well as shifts in seasonality (Bardgett et al., 1999; Steenwerth et al., 2006; Buckeridge et al., 2013) can alter the microbial community functions and composition. Many of these factors interact with each other and have both direct and indirect effects on the soil microbial community. For example, straw left on top of the soil would add organic carbon to the soil through decomposition, and it would reduce water evaporation (Carvalho et al., 2017). Moreover, the application of organic residues as fertilizer introduce not only organic carbon to the soil, but also mineral nutrients and, depending on the type of the residue, it may change substantially soil pH (Silva et al., 2014), which may counter the stimulatory effect of extra carbon input (Canellas et al., 2003).

Soil microbes are primary mediators of organic matter decomposition (Kuramae et al., 2013) and nutrient cycling (Rousk and Bengtson, 2014). Organic and inorganic fertilizer amendments are used to increase nutrient availability to plants, but they can also affect the soil microbial community and its functionality by directly or indirectly affecting the physical and chemical properties of soil. The application of organic and inorganic fertilizers may disturb microbial communities such that community members die or change their abundances (Rykiel, 1985; Suleiman et al., 2016). Disturbances are often classified as pulses or presses depending on their duration (Bender et al., 1984; Shade et al., 2012). In general, organic and inorganic fertilizer additions are pulse disturbances, they are relatively discrete, short-term events, whereas presses are long-term or continuous, such as liming, that change the soil pH. The soil microbial community may show to be resistant or resilient to the disturbances or if the community appears to be sensitive, it may perform differently (Figure 2) or appears to be functionally redundant. Resistance is defined as the degree to which a community is insensitive to a disturbance (Allison and Martiny, 2008) and resilience is the phenomenon that a community returns to its original composition after being disturbed (Allison and Martiny, 2008); commonly referred to as community recovery (Shade et al., 2012; Griffiths and Philippot, 2013). Finally, functional redundancy refers to the property that even when the community composition is sensitive and not resilient or resistant, its functions remain similarly to the original community (Allison and Martiny, 2008). The functionally redundant microbial community is related to the presence of functionally redundant species in the community. However, the concept of functional redundancy remains controversial (Shade et al., 2012). Thus, depending on the disturbance, duration and microbial community stability, the community's response can differ substantially.

The stability of microbial communities can be investigated in terms of functional or compositional parameters. If functions are carried out by many taxa (Schimel, 1995) changes in community composition may not lead to functional changes (Allison and Martiny, 2008). On the contrary, if functions are performed by few microbes, changes in the community composition may change these functions. Shade et al. (2012) analysed 378 studies of microbial responses to biotic and abiotic disturbances, in 82% of the cases the community appeared to be sensitive to the disturbance, 31% were changes in composition, 26% in functionality and 43% showed changes in both composition and function. Only a few studies measured resilience (Shade et al., 2012) and a small fraction, 23%, the community returned to the pre-disturbance condition, of which 56% in composition, 35% in function, and 9% to both. The authors also reported that microbial communities may be more resilient after short-term than after long-term disturbances. Besides recovery from short-term disturbances was reported by Shade et al. (2012) more often for the microbial community functionality than for the composition, while recovery from long-term disturbances was approximately the same for both function and composition.

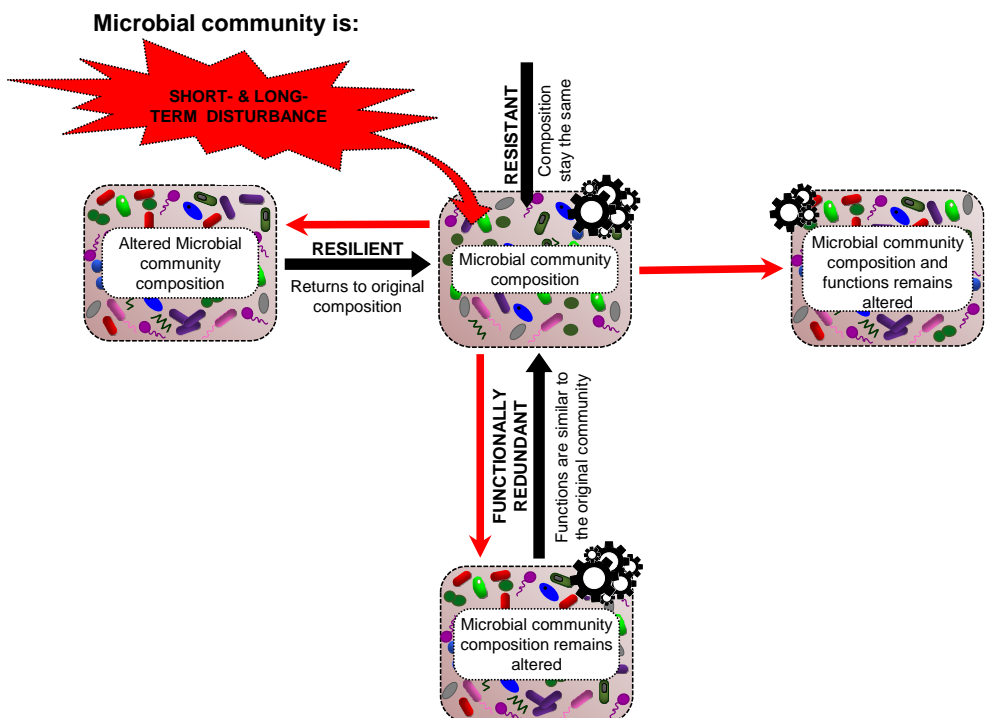


Figure 2 | Scheme of how disturbances can change microbial community composition and functions. Adapted from Allison and Martiny (2008).

Organic residues may differ in organic matter composition, for example C/N ratio, which affects the decomposition rate and the microbial community structure and function. For instance, the presence of labile organic components in

the organic residue promotes the growth of microorganisms with copiotrophic lifestyle that grow rapidly in nutrient-rich environments compared to organisms adapted to nutrient-poor conditions (oligotrophic lifestyle) (Navarrete et al., 2015a), while straw additions enhance cellulolytic microorganisms (Kuramae et al., 2013; Kielak et al., 2016b). Thus, application of inorganic or organic compounds on a short or long-term basis might result in positive, neutral or negative effects in soil microbial community structure (Biederbeck et al., 1996; Hu et al., 2011; Williams et al., 2013; Balota et al., 2014; Cassman et al., 2016; Suleiman et al., 2016). In general soil microbial communities are resilient to biotic disturbances and usually exclude successfully exotic organisms (Levine and D'Antonio, 1999). Suleiman et al. (2016) documented that pig manure used as fertilizers affected microbial functional diversity, and changed the microbial structure temporarily. The metabolically active microbial community was resilient recovering to its original status. Nevertheless, there is so far very little evidence of a connection between alterations on microbial community composition and function over time series after input of bioenergy organic residues.

Microbial community responses to pulse- and press-type disturbances are important to consider in the context of the sustainability of bioethanol production and global climate change. The organic residues produced during sugar and ethanol production, i.e. straw and vinasse do affect the microbial community structure (Navarrete et al., 2015a; Pitombo et al., 2015). Results of field studies have shown that different management strategies with straw (Huang et al., 2012) and vinasse (Navarrete et al., 2015a), alter the soil bacterial community composition. In general, straw application increases the microbial community metabolic activity (Navarro-Noya et al., 2013) and vinasse amendment causes positive or negative effects on specific microbial groups (Pitombo et al., 2015). Thus, understanding of how microbial communities and functions change over time after vinasse and straw applications is important to understand processes such as succession after or recovery from perturbations and so to assess the consequences of the use of these residues in tropical agricultural systems.

Also changes in climatic conditions through changes in water content and temperature are important factors regulating the composition and activity of microbial communities in soils (Bell et al., 2008). Thus, the responses of the soil microbial community to organic and inorganic fertilizers will be season dependent. For example in a rainy season the labile organic carbon input from organic fertilizers may be less important than in a dry season, due the larger decomposition of native soil organic matter under rainy conditions. Previous studies showed that water content plays an important role in the composition and diversity of microbial communities over seasons in environments such as sediments (Valverde et al., 2014), forest soils (Bouskill et al., 2013) and agriculture soil (Phillips et al., 2015). Low water content inhibits microbial activity by restricting substrate supply and selecting for only species adapted to survive under these conditions (Stark and Firestone, 1995; Valverde et al., 2014). The maximum aerobic microbial activity

occurs at moisture levels of around 70% of water holding capacity. Changes in temperature may also influence the structure of bacterial communities and temperature is positively correlated with microbial activity (Lipson, 2007). Seasonal variations in water content and temperature have considerable impact on important processes such as organic matter decomposition (Stark and Firestone, 1995; Karhu et al., 2014). However, it is only poorly understood how microbial communities respond to seasonal variations in moisture and temperature after application of mineral and organic residues. Few studies show that seasonality may affect the structure of microbial communities and functional properties, suggesting that microbial dynamics is influenced by seasonal variability (Smith et al., 2015). On the other hand, others studies showed that bacterial communities are not strongly tied to seasonal variations (Landesman and Dighton, 2010). The central-Southern region of Brazil, i.e. the most important region for sugarcane production, has two defined seasons, rainy summers with high temperature and dry winters with mild temperatures. Therefore, understanding the impact of seasonal variability in combination with fertilization on the soil microbial community will help to develop better strategies to optimize the use of mineral and organic fertilizers.

3. Greenhouse gas emissions

The increase in the concentration of greenhouse gases (GHG) in the atmosphere after the industrial revolution is one of the main problems causing global warming. Nitrous oxide (N_2O), carbon dioxide, (CO_2) and methane (CH_4) are the main GHG emitted due to anthropogenic activities. The global warming potentials of N_2O and CH_4 are 298 and 34 times greater than CO_2 (IPCC, 2013). In addition, N_2O is one of the main molecules that are responsible for the destruction of ozone layer (Ravishankara et al., 2009).

In Brazil, N_2O is the most important GHG emitted from sugarcane soils (Cerri et al., 2009). Recent studies showed that N_2O emissions from inorganic fertilizer are lower than reported by Crutzen et al. (2008). They claimed that 3 to 5% of the total N applied, and Lisboa et al. (2011) claimed 3.9% of N applied being emitted as N_2O from sugarcane fields (Vargas et al., 2014; Soares et al., 2015; Siqueira Neto et al., 2016). Such high N_2O emissions almost denied the use of sugarcane biofuel as an option to decrease GHG emission. However, other studies showed that the N_2O emission from sugarcane fields in Brazil ranged from 0.2 to 1% of applied N (Filoso et al., 2015) which is even lower than the default value of 1% of the N applied in the field (IPCC, 2013). These data suggest that sugarcane might be a sustainable alternative bioenergy source in terms of the reduction of GHG emissions as compared to fossil fuel (Boddey et al., 2008; Crutzen et al., 2008; Galdos et al., 2010). However, when vinasse was applied with N fertilizer, the emissions increased up to 3% of applied N (Carmo et al., 2013). Similar results were obtained by Pitombo et al. (2015), who found that the proportion of N emitted

as N₂O was 2.4% when vinasse and N were applied combined in the soil. Paredes et al. (2014) also examined the effect of vinasse and fertilizer application in a field experiment. The N₂O emission after application of inorganic N was 0.2%, but reached 0.6 and 0.7% when N was applied with vinasse with a difference of application timing over two days in the same area. The authors found similar results when vinasse was applied with a delay of 3 or 15 days related to the moment of inorganic fertilizer application; 0.77% and 0.78% of applied N was lost as N₂O (Paredes et al., 2015) against 0.58% of N applied when only inorganic N was applied. The results of N₂O emissions in literature are quite variable, but in most cases application of vinasse with mineral N in the same area increased N₂O emissions.

The high N₂O emissions observed in studies when vinasse is applied were assigned to the increase in soil microbial respiration (Carmo et al., 2013; Paredes et al., 2014; Paredes et al., 2015) and high water content (Barton and Schipper, 2001; Carmo et al., 2013). Barton and Schipper (2001) observed similar results on the increase of emissions of N₂O and CO₂ in soils that received inorganic N plus dairy farm effluent when compared to inorganic fertilizer applied with water. The authors impute these increased emissions to the larger organic C availability, higher soil water content and lower aeration resulting in depletion of O₂ in the soil, which stimulate the production of N₂O by denitrification.

Furthermore, the soil reactions that result in GHG emissions are affected by climatic conditions. The sugarcane harvest period in São Paulo State and in Central-Southern region of Brazil is between April and November, which covers three seasons, starting in the fall (April to June) and ending in the spring (October-December). In the early and mid-season (fall and winter) temperatures are moderate with long dry periods. However, at the end of the season (spring) the temperatures are higher with occurrence of rain, i.e. ideal conditions for high N₂O production by denitrification. Therefore, changes in temperature and moisture due seasonality and nutrient availability by application of vinasse and inorganic N may affect the structure and functionality of microbial communities including those involved in N-cycling. Thus, in order to assess the GHG emission factors it is necessary to take into the account the timing of the mineral fertilizer and vinasse application.

N₂O is produced in soil via biotic as well as abiotic process. The abiotic process, chemodenitrification, is based on chemical decomposition of hydroxylamine (NH₂OH), nitroxyl hydride (HNO) or nitrite (NO₂⁻) with organic and inorganic compounds at low pH (<4.5). The potential to biotic N₂O production has been observed in more than 60 bacterial and archaeal genera and more recently also in fungi N₂O production has been demonstrated (Hayatsu et al., 2008; Higgins et al., 2016; Hink et al., 2016). Production of N₂O in soils occur mainly due to the processes of nitrification and denitrification (Figure 3) (Stevens and Laughlin, 1998; Németh et al., 2014; Martins et al., 2015; Soares et al., 2016; Xu et al., 2017). In oxic soils, well-drained soils, typical for agricultural soils (<60% water-filled pore

space - WFPS), N₂O is mainly produced by organisms involved in the first step of nitrification, i.e., ammonium oxidation (bacteria and archaea) (Bollmann and Conrad, 1998; Bateman and Baggs, 2005; Baggs et al., 2010; Hink et al., 2016). However, under suboxic or anoxic conditions (60-90% WFPS), facultative heterotrophic denitrifiers (Tiedje et al., 1983; Di et al., 2014) dominate N₂O production.

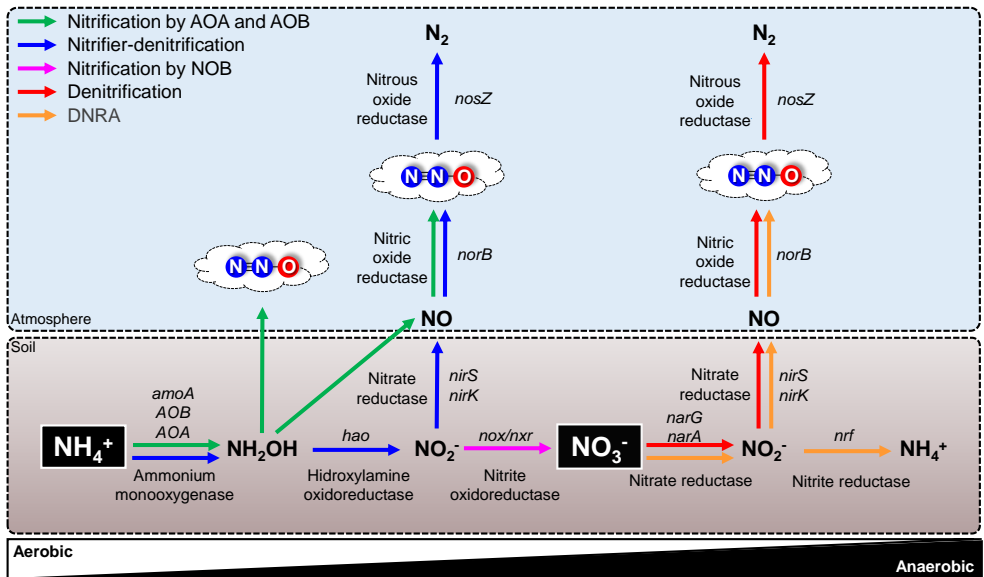


Figure 3 | Schematic diagram of the major microbial pathways of N₂O production in soils.

The multiple pathways include nitrification (ammonia oxidation performed by AOA and AOB and nitrite oxidation by NOB), nitrifier denitrification (performed by AOA and AOB), denitrification (heterotrophic denitrification by heterotrophic bacteria), DNRA (dissimilatory nitrate reduction to ammonium, by unknown microorganisms) and anammox (anaerobic ammonium oxidation, by anaerobic ammonia oxidizers). Enzymes: *amoA* (ammonia monooxygenase); *hao* (hydroxylamine oxidoreductase); *narG* (membrane-bound nitrate reductase); *napA* (periplasmic nitrate reductase); *nirK* (copper-containing nitrite reductase); *nirS* (cytochrome cd1 nitrite reductase); *nxr* (nitrite oxidoreductase); *norB* (nitric oxide reductase) *nosZ* (nitrous oxide reductase) and *nrf* (Nitrite reductase). Different microbial groups and pathways are indicated clearly by different colors. Adapted from Hu et al. (2015).

Nitrification is the aerobic oxidation of ammonia (NH₃) to nitrate (NO₃⁻) and it occurs in two phases mediated by autotrophic microorganisms (Figure 3). In the first phase ammonia-oxidizing bacteria (AOB) or archaea (AOA) oxidize NH₃ to nitrite (NO₂⁻), and subsequently NO₂⁻ is oxidized to NO₃⁻ by nitrite-oxidizing bacteria (NOB) (NO₂⁻ → NO₃⁻). The first phase (NH₃ → NH₂OH/HNO → NO₂⁻), i.e., ammonia oxidation, is catalyzed by the *amoA* gene encoding ammonia

monooxygenase. It is known to be present in β - or γ -proteobacteria (AOB) and the newly described *Thaumarchaeota* phylum (AOA). The *nxrB* gene encodes the nitrite oxidoreductase and regulates the second phase of nitrification. The first main N_2O -yielding pathway during nitrification occurs under aerobic conditions, N_2O emission from AOB results from the incomplete oxidation of NH_2OH to either nitroxyl (HNO) or NO (nitric oxide) (Smith and Hein, 1960; Hu et al., 2015) and subsequently N_2O is produced. Recently Caranto et al. (2016) demonstrated that another, direct enzymatic pathway from NH_2OH to N_2O at anaerobic conditions exists, and this pathway is mediated by cytochrome P460. The second N_2O -yielding route is named nitrifier denitrification and occurs at both high and low oxygen concentration. AOB possess machinery that reduces NO_2^- to N_2O via a nitric oxide (NO) intermediate (Ritchie and Nicholas, 1972; Shaw et al., 2006; Stein, 2011). Recently it has been found that nitrification can occur during a single step performed by bacteria of the *Nitrospira* genus (Daims et al., 2015; van Kessel et al., 2015); however, it is not yet known whether N_2O emission occurs in this one-step process.

Denitrification is a multistep reaction performed by a variety of bacteria and fungi. During denitrification oxidized mineral forms of N (NO_3^- and NO_2^-) are reduced to the gaseous products NO, N_2O and N_2 under oxygen-limited condition ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) (Figure 3). The sequential processes of bacterial denitrification are regulated by divergent reductases encoded by distinct functional genes; *narG* or *napA* genes encode nitrate reductase, *nirK* or *nirS* genes encode two entirely different types of nitrite reductase; *cnorB* or *qnorB* genes encode nitric oxide reductase and *nosZ* gene encodes nitrous oxide reductase (Philippot et al., 2007; Jones et al., 2013).

Despite considerable knowledge of the processes involved in N_2O production, most of the work was conducted under controlled conditions, thus in studies in which the impact of climatic conditions and variations during the year was not taken into account. The prevalence of the processes that control N_2O production in tropical soils during the growth of sugarcane has only begun to be addressed.

4. Research aims and thesis outline

The major goal of the research described in this thesis was to understand to what extent organic vinasse applications and sugarcane straw in combination with inorganic fertilizers affect the composition, functions and dynamics of the soil microbiome at seasonal climatic variations (Figure 4). Modern molecular techniques such as new generation sequencing were used to analyze microbial communities in field samples. N_2O production over time was also measured in the field and linked to data on microbial community structure and functioning.

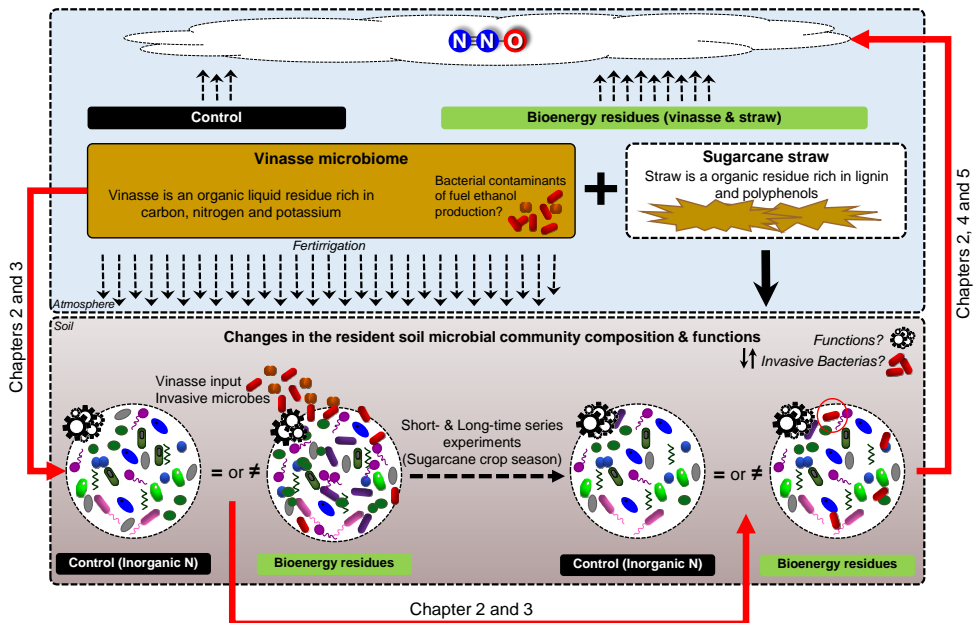


Figure 4 | Schematic overview of the chapters presented in this thesis.

The research questions addressed are:

- (i) To what extent are the composition and functionality of the resident microbial community in a sugarcane field affected by organic residue and inorganic fertilizer amendments (sugarcane straw, organic vinasse and inorganic nitrogen)?
- (ii) How do the single and combined applications of vinasse, straw and inorganic fertilizers influence N_2O emissions from soil?
- (iii) Is the microbial community resistant or resilient to a pulse disturbance brought about by the application of organic residues and inorganic fertilizers?
- (iv) How do climatic conditions affect the responses of the microbial community involved in N_2O production to disturbances?
- (v) Which microbial process, i.e. nitrification or denitrification, contributes most to the N_2O production?
- (vi) Do fungal denitrifiers contribute to N_2O production in tropical soils amended with straw?

This thesis starts with an assessment of how the soil microbial community's composition and functions are affected by bioenergy residues (organic vinasse and sugarcane straw) and inorganic fertilization and how these residues are linked with N_2O emissions. In *Chapter 2* a short-term sugarcane field experiment (crop season 2012/2013) is described that was designed to assess the changes in the soil microbial community composition and functions through time by analyzing shotgun metagenomics data and N_2O emissions.

In *Chapter 3*, the effect of organic vinasse and inorganic N fertilizer application on the resident soil microbial community was monitored during an

entire sugarcane crop season (season of 2014/2015) as well as CO₂ emission. This allowed for evaluating the stability and dynamics of the microbial community in response to perturbations. The microbial community was analyzed by PCR-amplified 16S ribosomal DNA. In addition, the microbes present in vinasse were tracked back into the soil and the potential invasiveness of those microbes was evaluated.

In *Chapter 4 and 5* investigations on the N₂O losses from sugarcane planted soils receiving different fertilization regimes (organic vinasse and inorganic N fertilizer) and the potential role of nitrification and denitrification processes in N₂O productions are described. In *Chapter 4* I studied how different seasons (spring-rainy/winter-dry, crop season 2013/2014 and 2014/2015, respectively) affected the N₂O losses from sugarcane planted soils receiving concentrated and non-concentrated vinasse. Furthermore, in this chapter I described the assessment of the abundance of microbial genes encoding proteins involved in the N cycle and N₂O production, such as archaeal and bacterial *amoA*, fungal and bacterial *nirK*, and bacterial *nirS* and *nosZ*. In *Chapter 5* I describe a study on the main microorganisms responsible for the N₂O production in soil after amendments of bioenergy crop residues.

Finally, in *Chapter 6* I combine the main observations described in this thesis and further discuss the role of bioenergy residues in the N₂O emissions from sugarcane production fields and the changes in the soil microbial community composition and functions. Here, I present a future outlook on the potential strategies to optimize the sustainable use of organic vinasse and inorganic N fertilizers in the sugarcane and ethanol production leading to low N₂O emissions.