THE UNIVERSITY OF ADELAIDE

Thi Huong Xuan Le

NITROGEN AND PHOSPHORUS REMOVAL FROM WASTEWATER ADDED TO SAND BY WHEAT STRAW ADDITION AND WHEAT PLANTS

Thesis submitted to the University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy

School of Agriculture, Food and Wine

Faculty of Sciences

The University of Adelaide – Waite campus

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Dedicated to my parents, husband and daughter

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ABSTRACT

Wastewater irrigation can add nutrients to soils, but also increase nutrient leaching, particularly in sandy soils. For sustainable use of wastewater, nutrient leaching should be minimized. It is unclear how wheat straw amendment to sand or wheat growth on sandy soil influences removal of N and P from wastewater. This thesis aimed to investigate (1) the ability of wheat straw to remove inorganic N and P from wastewater collected from a sewage treatment plant when mixed into sand at different rates (Experiment 1) and decomposition stages of the straw (Experiment 2), and (2) the effect of wastewater irrigation at different early growth stages of wheat plants on nutrient uptake (Experiment 3).

In the first experiment, wheat straw was mixed with sand at 2.5, 5, 7.5, 10, 12.5 g kg^{-1} in leaching columns before adding wastewater. The control was unamended sand. Leaching was conducted on 4, 8 and 16 days after wastewater addition. With straw amendment, nitrate in the sand-straw mixes was lower than in sand alone while ammonium was higher at 12.5 g straw kg^{-1}. Over 95 % of inorganic N from added wastewater was removed irrespective of straw rate. Straw amendment had no consistent effect on P leaching.

In the second leaching column experiment, sand was mixed with wheat straw at 12.5 g straw kg^{-1} and incubated moist for 7 or 14 days or added just before adding wastewater (fresh straw). The control was unamended sand. Leaching was conducted 4, 8 or 16 days after wastewater addition. With straw amendment, available N in the sand-straw mixes was highest in fresh straw on day 16. Leachate inorganic N was much lower than in sand alone irrespective of straw decomposition stage.

In both leaching column experiments, very little N_2O was released, suggesting that denitrification was not an important process. Likely mechanisms for nutrient removal by straw are dissimilatory nitrate reduction to ammonium and nutrient binding to straw.

It was concluded that mixing wheat straw into sandy soil prior to wastewater application can substantially reduce inorganic N leaching.

In a pot experiment, sandy soil was left unplanted (control) or planted with wheat, which was grown for 7, 14 or 21 days before wastewater addition. All pots received reverse osmosis (RO) water for 20 days. Half of the planted pots and unplanted pots were irrigated with wastewater from day 21 to 35, the other pots still received RO until day 35. Wastewater irrigation increased N uptake compared to RO irrigation only in plants that were 21 days old before wastewater addition but had little effect on plant growth and on inorganic N and P in soil. However, presence of wheat reduced available N and P in soil compared to unplanted soils which would reduce potential of nutrient leaching after wastewater irrigation.

It can be concluded that inclusion of organic amendments and/ or suitable crops are the potential options for wastewater reuse on sandy soils. Field experiments should be carried out to confirm the applicability of these effects.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Thi Huong Xuan Le

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LIST OF PUBLICATIONS

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Leaching of nutrients is a major environmental concern worldwide. It occurs when mobile nutrients in the soil solution percolate through the soil profile and move beyond the rooting zone where they are unavailable for plants (Major et al., 2012). Leaching affects nutrient cycling in agriculture (Treat et al., 2016). It can lead to a decline in soil fertility and acceleration of soil acidity (Laird et al., 2010; Lichtenberg & Shapiro, 1997), reduction of crop yield, increased production costs (Tirado & Allsopp, 2012), and water pollution (Parris, 2011).

Leaching of nutrients is more of a problem in sandy soil than other soil types because sandy soils have a number of properties that enhance nutrient loss such as coarse texture, low fertility and low capacity for holding water and nutrients (Farrington & Campbell, 1970; Mtambanengwe & Mapfumo, 2006). Research is needed to inform development of management strategies which minimise nutrient leaching from sandy soils.

The direct or indirect discharge of untreated wastewater to receiving water bodies can result in deterioration of aquatic ecosystems causing environmental hazards such as toxic algae (Avnimelech et al., 1993). During wastewater treatment, physical (e.g., adsorption, and filtration), chemical (e.g., chemical precipitation and ion exchange), and biological (e.g., plant uptake and microbial degradation) techniques have been employed for nitrogen (N) and phosphorus (P) removal (Ma et al., 2011). Among these techniques, biological methods and adsorption have been commonly used and shown to have high nutrient removal efficiency (Wang et al., 2016b).

Organic amendments have potential for reducing nutrient leaching particularly in sandy soils, because organic materials increase water and nutrient holding capacity through improving soil aggregation, porosity, pore size distribution and nutrient content (Gupta et al., 1977). Compared to other organic materials, wheat straw is a low cost and widely available agricultural by-product (Liu et al., 2013b; Soares & Abeliovich, 1998). Wheat straw has been shown to retain nutrients by binding on functional groups of cellulose, hemicelluloses and lignin (Gao et al., 2016). It is an effective adsorbent for binding cations including ammonium, phosphorus and heavy metals (Farooq et al., 2010; Jassal et al., 2015; Todorciuc et al., 2015).

There is increasing trend on recycling of treated wastewater by disposal on agricultural land, particularly in developing countries or in semi-arid and arid zones (Avnimelech et al., 1993; Jalali et al., 2008). Wheat straw amendment to sandy soil has high potential in removing N and P from wastewater. This is because wheat straw provides a carbon rich substrate for microbial activities such as denitrification (Ashok & Hait, 2015; Warneke et al., 2011), which converts nitrate to N_2 gas or in dissimilatory nitrate reduction to ammonium where nitrate is reduced to ammonium (Burgin & Hamilton, 2007; Rezvani et al., 2017; Sander et al., 2015) under anaerobic conditions.

The literature review will cover the following topics: (1) factors controlling nutrient leaching; (2) causes of nutrient leaching and the environmental consequences; (3) role of organic amendments in reducing leaching and mechanism of nutrient retention.

1.2. Nutrient leaching from soils and implications for the environment

Nutrient leaching is defined as downward movement of available nutrients out of the surface soil layer and rooting zone with percolating water (Taylor & Parkinson, 1988). The leaching risk of a nutrient is largely influenced by its concentration and mobility in soil (Likens et al., 1969). The mobility of nutrients is commonly accelerated in intensive agriculture where often high rates of fertilisers are used, sometimes combined with irrigation. High concentrations of N and P in the soil solution have been found in agricultural fields (Laird et al., 2010).

The anions nitrate and phosphate in the soil solution have unique properties in terms of chemical and biological reactions which affect their production and mobility. The mobility of nitrate is mostly regulated by biological processes whereas that of phosphate is strongly affected by adsorption and precipitation reactions. The positively charged ammonium ion (NH_4^+-N) can be bound to negatively charged soil particles, such as clay (Jellali et al., 2011; Wang et al., 2015). Nitrification is the microbial conversion of ammonium to nitrate and H^+ (Johnson & Cole, 1980). Nitrate (NO_3^-) is readily leached through the soil because it is a negatively charged ion and therefore repelled from soil particles which have predominantly negative charge (Shirmohammadi et al., 1991). Phosphate (PO_4^{3-} or HPO_4^{2-}) is also negatively charged, but readily binds to aluminium, iron, and calcium which are, in turn bound to organic matter and clay minerals (Peng et al., 2011). Therefore, the phosphate concentration in the soil solution and percolating water is usually low. However, phosphate can also reach aquatic ecosystems through soil particle movement, e.g. by surface erosion (Tirado & Allsopp, 2012).

Nutrient leaching can lead to a decline in soil fertility and acceleration of soil acidity (Laird et al., 2010; Lichtenberg & Shapiro, 1997), reduction of crop yield, increase of production costs and water contamination (Tirado & Allsopp, 2012). Accumulation of nitrate and phosphate in freshwater and marine ecosystems can lead to excessive growth of photosynthetic aquatic microorganisms (Karaca et al., 2004). Algal blooms can result in oxygen deficiency and algal toxins in drinking water causing health hazards and massive fish and shrimp kills (Corrales & Maclean, 1995).

Nutrient leaching is affected by various natural and anthropogenic factors such as climate, hydrology, soil, topography, land use, fertilisation and cultivation (Burt et al., 1993; Ekholm et al., 2000; Vagstad, 2001). In particular, nutrient leaching is related to soil properties such as soil texture, structure, pH and organic matter (Hillel, 2008; McCauley et al., 2009). Soils with a sandy texture, low organic matter content and low water-holding capacity are particularly susceptible to leaching (Russell, 1995) as will be discussed in more detail below.

1.3. Soil factors affecting nutrient leaching

1.3.1. Soil texture

Soil texture refers to a relative proportion of sand, silt and clay particles which affect water and nutrient retention capacity of a soil (Hillel, 2008). Sand particles are larger than clay or silt particles and mainly consist of quartz which has a very low surface charge. Therefore, they have a low specific surface area (Hamarashid et al., 2010) and low cation exchange capacity (CEC) and low capacity for holding water and nutrients (McKenzie et al., 2004).

The dominance of sand particles and large pores within sandy soils allow water and nutrients to move rapidly through the soil (Farrington & Campbell, 1970). In contrast, heavy clay-textured soils contain a large proportion of clay particles and small pores which hold more water and nutrients. Therefore, water movement is much slower than in lighter texture soils (Hillel, 2008).

Soil texture affects NO_3^--N retention. Soils with high proportion of silt, clay and organic matter retained more NO_3^--N than sandy soils (Gaines & Gaines, 1994). In a six-year lysimeter study, Webster et al. (1986) showed that average N leaching from sandy loam was nearly two-fold higher than from clay soils. Similarly, Kolenbrander (1981) indicated that nitrate loss was two-fold higher in sandy soils than clay soils when mineral N applications were not higher than 100 - 200 kg N ha^{-1}.

Compared to N leaching, effects of soil texture on P leaching are more complicated depending on P sorption and desorption capacity of soil particles and landscape. Surface runoff of soil particles with P attached is common in hilly areas. In flat regions on the other hand, P transport through the soil profile is the dominant mechanism for P loss (Tunney et al., 1997).

1.3.2. Soil structure

Soil structure refers to the size, shape and the arrangement of soil particles (sand, silt, and clay) and voids into groupings called aggregate or peds (Letey, 1991). Soil aggregates form distinct patterns within soil horizons (Lavelle & Spain, 2001). Soil structure is also an important indicator of water storage capacity of a soil because it influences pore size distribution (Djajadi et al., 2012). Loosely packed soil particles increase water movement through the soil, whereas tightly compacted soil particles store more water and reduce water movement through the soil

(Lavelle & Spain, 2001). Sandy soils have poor soil structure, as the low clay and silt content limits aggregation (Djajadi et al., 2012).

Soil water retention capacity is influenced by total porosity and pore size distribution (Nimmo, 2004). Water is held more tightly in smaller pores than large pores (Nimmo, 2004). Large pores dominate in sandy soils, whereas medium to small pores are dominant in finer textured soils. Therefore, drainage is more rapid in sandy soils than finer textured soils. This, together with low nutrient binding capacity leads to nutrient leaching in sandy soils. However, the presence of large cracks and macropores in clay soils during dry periods may result in bypass flow which contributes the rapidly vertical movement of water after rain or irrigation (Brown et al., 1995).

1.3.3. Soil pH

Soil pH is an indicator of soil acidity or alkalinity (McCauley et al., 2009). It is one of the most important parameters affecting leaching of both inorganic and organic constituents (Fruchter et al., 1990; Mudd et al., 2004), because it affects nutrient solubility, chemical transformations, nutrient binding to soil colloids and microbial activity (Marschner, 2011). Soil pH near 7 is optimal for the availability of most nutrients (McCauley et al., 2009). The availability of most macronutrients (N, P, K, Ca, Mg and S) is highest within a pH range of 6.5 - 8, whereas the majority of micronutrients (B, Cu, Fe, Mn, Mi and Zn) are more available within a pH range of 5-7. According to Haynes and Swift (1986), increasing soil acidification accelerates cation leaching. Leaching loss is in the following order: $Ca^{2+} > Mg^{2+} > K^{+} > Na^{+}$. The availability of most micronutrients (Fe, Zn, Ni, Mn, Cu) in alkaline soils (pH 8) is low due to their stronger binding to soil and formation of minerals; in acidic soils they are more available which can lead to metal toxicities for crops (Lucas & Davis, 1961; McCauley et al., 2009).

Nitrification is inhibited at low pH, at pH < 5 NO_3^- concentration in the soil solution is low whereas that of NH_4^+ is high (Lucas & Davis, 1961). Liming of soils with pH 4.3 or 3.4 increased nitrification four-fold (Turk, 1939).

Phosphorus availability is highest at pH 6 - 7 (McCauley et al., 2009). However, its availability is low in alkaline and acidic soils. In alkaline soils, the most dominant forms of phosphate are HPO_4^{2-} and PO_4^{3-}. These phosphate ions precipitate with calcium and form calcium phosphate (Hinsinger et al., 2009). However in acidic soils, orthophosphate ($H_2PO_4^-$) dominates which reacts with aluminium and iron and becomes less available (Zheng, 2010).

1.3.4. Soil organic matter

Soil organic matter (SOM) is an important soil factor affecting nutrient leaching (Teklay et al., 2006). It acts as a soil conditioner improving water retention (Olness & Archer, 2005), infiltration (Pachepsky & Rawls, 2003), aggregation (Franzluebbers, 2002) and reducing compaction, crusting, runoff and erosion (Lal, 2004). Physically, SOM improves soil structure by stabilizing soil aggregates. Decomposed SOM and microbial metabolites cement soil particles together to produce stable aggregates (Anderson & Domsch, 2010; Brady & Weil, 2002). Chemically, SOM improves soil pH buffering and cation exchange capacity, providing a nutrient source for plants and soil microbes (Madejón et al., 2001).

Compared to sandy soils, clay soils typically have a higher organic matter content because organic matter can be bound to clay via cation bridges and then becomes less accessible for microbes (Tisdall & Oades, 1982). Furthermore, organic matter in clay soil is protected in macroaggregates against microbial decomposition (Rice, 2005).

In addition to soil factors, anthropogenic activities can contribute to nutrient leaching including overuse of chemical fertilizers, over-irrigation and wastewater irrigation.

1.4. Anthropogenic causes of nutrient leaching and environmental consequences

1.4.1. Overuse of fertilizers

Modern agriculture has increased crop yield but can also lead to overuse of both mineral and organic fertilizers (Zheng et al., 2013). The increasing input of fertilizer in farming systems is ascribed to the development of high-yielding crop varieties and the reduction in prices of chemical and fertilizer (Yadav et al., 1997). Global consumption of fertilizers has increased four-fold between 1950 and 1970. In the United States, the application of N, P and K in agriculture increased three to four times between 1960 and 1990 (Vroomen, 1989). In many greenhouse vegetable production systems in China, more than 3000 kg N ha^{-1} are applied annually which far exceeded plant requirement and less than 10% of applied N fertilizer is taken up by the growing crops (Ju et al., 2011). A significant proportion of fertilizer is lost from agricultural fields by leaching (Kirchmann et al., 2002; Raun & Johnson, 1999; Webster et al., 1986). This increases environmental problems and the risk to food safety. For example, the nitrate concentration of the leachate in greenhouse vegetable production systems in China ranged from 100 to 289 mg L^{-1} far exceeding the WHO standard of drinking water (50 mg L^{-1}) (Song et al., 2009). Similarly, over application of P fertilizers in both organic and mineral forms can build up P in soil profile (Zheng et al., 2013) and increase potential of P leaching. Therefore, excessive application of fertilizers to maximize short-term crop yields not only causes economic loss due to the loss of unused fertilizer but also induces groundwater pollution (Kirchmann et al., 2002; Perry et al., 1988; Zhang et al., 2013).

In order to minimize the negative effects of overuse of fertilizer and maximize fertilizer use efficiency, optimal fertilization techniques have been developed. Factors considered include application rate and method, time of fertilization, and fertilizer formulation (Li et al., 2018). For example, an application rate below the economically optimal rate (Schroder et al., 1998) combined with a variable deficit irrigation scheduling regime (Sexton et al., 1996) is a promising technique to reduce NO_3^- leaching (Gheysari et al., 2009) with limited effect on yield.

1.4.2. Over-irrigation

Development of efficient irrigation systems for modern agriculture is critical to increase global food supply in the context of an expanding population and growing food demand (Omezzine & Zaibet, 1998). About one-sixth of farmland worldwide receives irrigation to produce one third of global crops, so the yield of irrigated land is on average two-fold higher than of rain-fed land.

Irrigation practices greatly contribute to reducing food prices and increasing employment (Stockle, 2001).

An efficient water regime considers the actual requirement of plants, soil evaporation and soil water content (Tyler et al., 1996). However, over-irrigation is common, for example where flood irrigation is applied far exceeding the water requirements of crops, leading to saturated soils (Li et al., 2018). If over-irrigation is applied to soils with a low percolation rate (De Bruyn, 1982), water logging can occur causing changes in soil structure and reduction in crop productivity. According to De Bruyn (1982) cotton lint yields were highest only if plants were not exposed to over-irrigation during the growing season. Similarly, shoot fresh weight and total leaf area of tomato plants after four weeks of over-irrigation was decreased compared to well-drained plants (Fiebig, 2014).

Inefficient irrigation regimes can also lead to several environmental problems, especially nutrient leaching. The effect of over-irrigation on leaching and water quality may be greater than of fertilization (Li et al., 2018). Over-irrigation combined with poor drainage can lead to rising groundwater tables which potentially brings salts to the upper layers of the soil profile, resulting in soil salinity (Kijne, 1998). But in coarse textured soils, excess irrigation results in deep percolation to groundwater aquifers (Klocke et al., 1993). Therefore, high concentrations of both macro and micro nutrients are commonly found below the root zone of extensively irrigated areas (Li et al., 2018).

Nutrient leaching caused by over-irrigation can be minimized by improved irrigation techniques to meet the actual water requirement of the plants without affecting crop yields such as drip irrigation, optimizing irrigation frequency and reducing irrigation volume (Migliaccio et al., 2010).

1.4.3. Wastewater irrigation

Wastewater derived from anthropogenic activities is an environmental concern worldwide (Bedessem et al., 2005; Gibert et al., 2008). Wastewater generated from domestic, industrial and commercial activities has increased with population and economic development (Qadir et al., 2010).

Wastewater contains macro (C, N, P, K) and micro nutrients (Ca, Mg, B, Fe, Mn or Zn) depending on the source of effluent (Barreto et al., 2013; Liu & Haynes, 2011). Nitrogen is dominant in wastewater generated by agricultural activities (Boyer et al., 2002), while P is mostly derived from industrial and residential activities (Ruzhitskaya & Gogina, 2017). The most prevalent forms of N in wastewater are ammonium (NH_4^+-N), nitrate (NO_3^--N) and organic N (Sedlak, 1991; Sotirakou et al., 1999). The main forms of P in wastewaters are orthophosphates, polyphosphates and organic compounds (Sotirakou et al., 1999).

Reuse of wastewater for irrigation of cropland is a common practice worldwide, especially in developing countries where technologies for wastewater treatment are limited (Castro et al., 2013) or in semi-arid and arid zones where fresh water supply is limited (Avnimelech et al., 1993; Jalali et al., 2008). Many small-scale farmers in urban areas of developing countries use wastewater to irrigate edible crops for urban markets (Qadir et al., 2010). Major European cities and cities in the United States developed wastewater disposal schemes in agricultural fields in the 20th century (Asano & Levine, 1996; Cooper, 2007; Drechsel et al., 2010). According to the FAO, approximately 10% of the total irrigated land areas on Earth covering 20 million hectares in 50 countries receives partially treated or untreated wastewater (Cooper, 2007).

Wastewater can be a source of nutrients for plant growth (Siebe & Cifuentes, 1995). Wastewater irrigation can improve soil physical properties and supply nutrients for plant growth (Castro et al., 2013) and increase metabolic activity of soil microorganisms (Meli et al., 2002). Therefore, irrigation with reclaimed wastewater can increase crop yield (Meli et al., 2002). For example, wastewater irrigation in the Tula Valley in Mexico annually provided 2400 kg organic matter, 195 kg N, and 81 kg P per ha, significantly increasing crop yields (Jimenez, 2005).

Wastewater irrigation, however, can lead to environmental degradation due to leaching (Castro et al., 2013). Long term wastewater application can add metals to soils and increase the mobile and easily mobilizable metal fractions (Siebe & Cifuentes, 1995). Nitrogen and P accumulate in the runoff or leach into the groundwater resulting in deterioration of lakes and natural water bodies because of toxic algae bloom (Avnimelech et al., 1993; Castro et al., 2013; Howarth et al., 2002). In addition, irrigation with wastewater can promote salinity and sodicity of soil and shallow groundwater because sodium (Na^+) in wastewater replaces soil exchangeable cations (Ca^{2+}, Mg^{2+}, K^+) which then leach to aquatic ecosystems (Jalali et al., 2008). Wastewater application to sandy soils is particularly problematic because sandy soils have low water holding capacity, low specific surface area for adsorption (Hamarashid et al., 2010), and low cation exchange capacity. To mitigate the harmful effects of wastewater irrigation, treatment of wastewater prior to irrigation, and alternative irrigation and water management practices are essential (Castro et al., 2013; Gardenas et al., 2005). Prior to irrigation, nutrients in wastewater can be removed in wastewater treatment plants (Daifullah et al., 2003) or by adding organic amendments to soil that can bind or transform nutrients in the wastewater (Yao et al., 2012). However, nitrate is difficult to remove as is discussed further below.

1.5. Organic materials for nutrient retention

1.5.1. Types of organic materials

Organic materials have been widely used as adsorbents to remove pollutants from aqueous solutions (Bellahsen et al., 2018). They have several important properties essential for nutrient retention (Bellahsen et al., 2018) such as high surface area, porosity and long life (Shon et al., 2006). In addition, some agricultural residues have a high number of functional groups (e.g., –OH, –COH) in their cellulose, hemicellulose and lignin components. These functional groups have ion-exchange capacity and general adsorptive characteristics, therefore they can enhance condensation, etherification and polymerization of nutrients in the wastewater (Bellahsen et al., 2018).

Compared to other agricultural residues, wheat straw is relatively low cost and widely available (Liu et al., 2013; Soares et al., 1998). Cellulose, hemicelluloses and lignin (representing about 30, 30, and 15% of total organic C) in wheat straw have a large number of functional groups

(Gao et al., 2016) that can bind cations such as NH_4^+ (Jassal et al., 2015). Wheat straw has been used for nutrient retention from wastewater. For example, wheat straw was used to remove metal ions in wastewater (Farooq et al., 2010; Todorciuc et al., 2015), ammonium-N from cattle urine (Nimenya et al., 2000) and as a biofilm supporter for denitrification of synthetic wastewater under anaerobic conditions (Fan et al., 2012).

Other organic materials also have been used in wastewater treatment, for example woodchips (Christianson et al., 2017; Halaburka et al., 2017) and biochar (Bock et al., 2016; Yang et al., 2018). Woodchips have been used for bio-filtration systems to bind nutrients with removal of about 80% of total N and P added with wastewater (Choudhury et al., 2016; Ruane et al., 2011). Biochar has a high capacity for sorption and has been used as amendment to sandy soil to regulate nutrient bioavailability in soil (Xu et al., 2013) and leachate composition (Yao et al., 2012). However nutrient retention capacity of biochar depends on its properties (Wang et al., 2016a) and nutrient types (Hale et al., 2013; Yao et al., 2012). Yao et al. (2012) tested 13 biochar types most of which sorbed little nitrate or phosphate. Only the biochar made from Brazilian pepperwood reduced nitrate, ammonium, and phosphate in the leachates by about 30% compared to soil alone. Production of biochar requires external energy inputs and specialist equipment, which is not widely available, particularly in developing countries.

1.5.2. Mechanisms of nutrient retention by organic materials

Adsorption is defined as a process wherein a material is concentrated on a solid surface from its liquid or gaseous surroundings (Gupta et al., 2009). The adsorption process is highly dependent on the properties of the adsorbent (e.g. surface area, pore structure and particle size), and of the adsorbate (e.g. solubility, molecular structure) and the properties of the solution (e.g. temperature, pH, availability of competing organic or inorganic substances) (Shon et al., 2006). There are four main steps in the adsorption process (Figure 1).

+ Step 1: The solute is transferred from the liquid to adsorbent's boundary layer.

+ Step 2: The solute is transferred through the boundary layer to the surface of the adsorbent.

+ Step 3: The solute diffuses from surface of the adsorbent to active binding sites.

+ Step 4: Sorption of the adsorbate to the solid phase, which is controlled by two primary driving forces.

i. The first driving force is related to solvent hydrophobic or hydrophilic properties which influence the intensity of adsorption process.

ii. The second driving force is the attraction of the solute to the solid. This includes two types of attraction: physical adsorption and chemisorption (Worch, 2012). The former is the attractive forces between the solid surface and the adsorbed molecules called van der Waals forces (in the absence of electrical repulsion) or coulombic (in the presence of electrical attraction). Chemisorption is influenced by the attraction forces between adsorbed molecules and the solid surface through chemical bonding (Gupta et al., 2009).

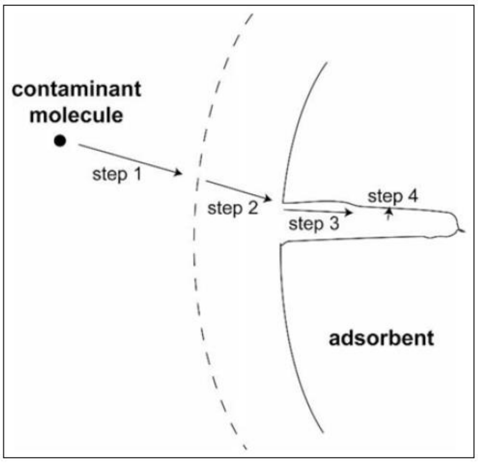

Figure 1. Steps in adsorption of a solute to a solid (Sotelo et al., 2013)

Decomposition of organic material leads to several changes in its chemical properties. This can influence nutrient availability by releasing compounds that can bind to soil particles. In the initial stages of decomposition, water-soluble components are rapidly depleted, resulting in high respiration rates (Summerell & Burgess, 1989). Later, hemicellulose and cellulose and other complex compounds are mineralized (Cogle et al., 1989; Reinertsen et al., 1984). During the first two weeks of straw decomposition, high proportions of organic acid anions are released (Lynch, 1978). These organic acids can influence nutrient availability by competing with nutrients for binding sites on soil particle surfaces (Jalali, 2009). In addition, C-containing functional groups change during wheat straw decomposition, particularly O-alkyl C to alkyl C and aromatic to COO/N-C=O groups (Gao et al., 2016). These changes in functional groups can affect binding of cationic and anionic nutrients.

1.5.3. Organic materials in wastewater treatment plants and effluent treatment

A typical wastewater treatment plant is divided into four main treatment sections including preliminary, primary, secondary and tertiary treatment. In the preliminary treatment, coarse solids (> 0.01mm) are removed in quiescent basins. Primary treatment continues removing the majority of suspended solids through clarifiers or sedimentation tanks. The following secondary treatment uses aerobic biological processes to substantially degrade the biological contents of the wastewater such as human waste, food waste, soaps and detergent. Secondary treatment systems are classified as fixed-film or suspended-growth systems (EPA, 2004). Finally, tertiary treatment improves effluent quality before it is discharged to the receiving environment (Shon et al., 2006). The tertiary treatment stage includes physical, chemical and biological methods (Ma et al., 2011).

For nitrate removal, reverse osmosis ion exchange, electron-dialysis and activated carbon adsorption (Feleke & Sakakibara, 2002; Islam & Suidan, 1998; Schoeman, 2009) have been applied. However, these methods have several disadvantages due to high cost and generated by-products (Ghafari et al., 2008). Biological nitrate removal from wastewater is a more economical alternative. In this process, organic materials provide the organic carbon source for microbial activity and serve as electron donors, with nitrate as the terminal electron acceptor for the oxidation of organic matter. Nitrate in wastewater can be converted into a number of N forms by different biological pathways such as denitrification or dissimilatory nitrate reduction to ammonium (Ashok & Hait, 2015; Burgin & Hamilton, 2007; Liu et al., 2013a).

To control the NH_4^+ concentration in wastewater, methods such as chemical precipitation, ion exchange, supercritical water oxidation, microwave radiation, biological treatment, and adsorption techniques have been used (Lin et al., 2009; Miladinovic & Weatherley, 2008; Segond et al., 2002; Shi et al., 2013; Uludag-Demirer et al., 2005; Vassileva et al., 2009). Among these methods, adsorption is considered a reliable and effective treatment which has economical advantages because it requires low energy input and easy operation (Ma et al., 2011).

Physical (e.g., adsorption, and filtration), chemical (e.g., chemical precipitation and ion exchange), and biological (e.g., plant uptake and microbial degradation) techniques have been employed for P removal (Ma et al., 2011). Among these techniques, adsorption provides high efficiency in P removal (Wang et al., 2016b). However, the success of this technology depends on an appropriate adsorbent (Onyango et al., 2007).

Organic materials have been reported as remediation option for treatment of wastewater. Fan et al. (2012) showed that wheat straw can be used as biofilm supporter for denitrification of synthetic wastewater with 100% nitrate removal within 15 days. Lowengart et al. (1993) suggested that when N-rich irrigation wastewater passed through a column of wheat straw with high C/N ratio, N immobilization is expected to occur at the beginning, while a sequence of nitrification and denitrification would take place later. Two types of sorbents made from rice husk have shown to be effective sorbent materials to remove heavy metals (Fe, Mn, Zn, Cu, Cd and Pb) in small wastewater treatment plants with removal efficiency up to nearly 100% (Daifullah et al., 2003). In acid sulfate soils, amendment with eucalypt and wheat biochars reduced leaching of protons and metals (Dang et al., 2016). In acid mine drainage, winery waste provided carbon and energy for sulphate-reducing bacteria to remove sulphate (>90%), Fe (61–91%), Zn, Cu (97%) (Costa et al., 2009).

1.5.4. Organic amendments for nutrient retention in soils

Organic amendments can increase soil water holding capacity and thereby improve nutrient retention. This is because organic amendments improve soil structure by reducing bulk density, increasing total porosity, therefore enhancing aggregate stability and pore space for water and nutrient retention (Adams, 1973). For example, composted amendments reduced bulk density from 1.6 to 1.4 g cm^{-3} and improved water holding capacity by up to 35% compared to the unamended control (Ozores-Hampton et al., 2011). Furthermore, organic amendments increase SOM that has a high surface areas for binding nutrients and holding water (Shon et al., 2006).

Organic amendments contribute to nitrogen retention and cycling in soil by regulating a number of biological pathways. Amendment of soil with organic residues results in a marked increase in the amounts of organic carbon which promotes two competing nitrate reduction pathways, namely dentrification (DNF) and dissimilatory nitrate reduction to ammonium (DNRA) (McCarty & Bremner, 1992; Rahman et al., 2019). They are both anaerobic microbial processes that compete for nitrate and nitrite in the environment where nitrate acts as the terminal electron acceptor for the oxidation of organic matter (van den Berg et al., 2016). In DNF, nitrate is reduced to N_2 by heterotrophic bacteria causing N loss to the atmosphere as N_2O and N_2 (Ashok & Hait, 2015; Warneke et al., 2011). In DNRA on the other hand, nitrate is converted to NH_4^+ which is available for microbial and plant uptake or can then be bound to the negatively charged clay minerals; therefore N is retained in the environment (Silver et al., 2001).

Both DNF and DRNA require low redox potential, available NO_3^- and labile C (Burgin & Hamilton, 2007; Zumft, 1997). However, the ratio of organic carbon to nitrate is an important factor that often determines the partitioning between DNF and DNRA (Burgin & Hamilton, 2007). DNRA is expected to be favoured by a high ratio of available C to electron acceptors (Tiedje et al., 1983) whereas denitrification is favoured at intermediate organic C availability (Thamdrup & Dalsgaard, 2002) and increased with decreasing residue C/N ratio (Aulakh et al., 1991).

1.6. Conclusion and research gaps

Nutrient leaching can have detrimental effects on the environment and crop production (Laird et al., 2010; Tirado & Allsopp, 2012), particularly in sandy soils, which have low water and nutrient holding capacity (Farrington & Campbell, 1970; Mtambanengwe & Mapfumo, 2006).

In developing countries, untreated or poorly treated wastewater may be applied to water bodies and soils (Castro et al., 2013). In developed countries, there is increasing trend to recycle treated wastewater with final disposal on agricultural land (Jaramillo & Restrepo, 2017). Irrigation with wastewater has been shown to supply nutrients for crop growth (Siebe et al., 1995). However, application of wastewater for farming can lead to water pollution due to leaching into surface or underground water (Howarth et al., 2002). Organic amendment is a potentially useful and cost-effective method to reduce nutrient leaching, particularly in sandy soils since organic materials derived from agricultural by-products are widely available at low cost (Liu et al., 2013b; Soares & Abeliovich, 1998). It has been shown that wheat straw is an efficient nutrient absorbent (Gao et al., 2016). Further, wheat straw is a carbon source for microbes involved in transformation processes such as biological denitrification (Ashok & Hait, 2015) and dissimilatory nitrate reduction to ammonium (Burgin & Hamilton, 2007). However, little is known about the influence of application rate of wheat straw as soil amendment or its decomposition stage before application of wastewater on nitrogen and phosphorus removal from wastewater. Further, influence of growing plants on available N and P in soil after irrigation with wastewater is unknown.

Therefore, the following research gaps will be addressed in this thesis

1. Can wheat straw addition to sandy soil increase N and P removal from applied wastewater compared to sand alone?
2. Does N and P removal increase with straw rate and do leachate composition and soil retention change with straw incubation time?
3. Is N and P removal greater with fresh straw than with pre-decomposed straw?
4. Does irrigation with wastewater increase growth of wheat plants compared to freshwater irrigation?
5. Can wheat plants growing on sandy soil irrigated with wastewater reduce N and P availability in soil?

Therefore, the present study has following aims:

1. Investigate the effects of different addition rates of wheat straw on inorganic N and P in leachate and nutrient availability in sandy soil after exposure to wastewater for different lengths of time (Chapter 2).
2. Assess the influence of decomposition stages of the straw and duration of contact with wastewater on inorganic N and P in leachate and the straw - sand mix (Chapter 3).
3. Determine the influence of wastewater application on wheat growth and nutrient availability in sandy soil (Chapter 4).

The thesis also includes the initial experiment which investigated the effect of the mixing of organic materials differing in C/N ratio on nutrient leaching in sandy soil (published as (Le & Marschner, 2018). However, the results were not consistent. Therefore, this topic was replaced by investigating the effects of organic amendment and plant growth on nutrient retention and leaching after wastewater application to sandy soil which is the main topic of this thesis. The paper of the initial experiment is shown in the appendix.

An outline of the design of the three main experiments is given below.

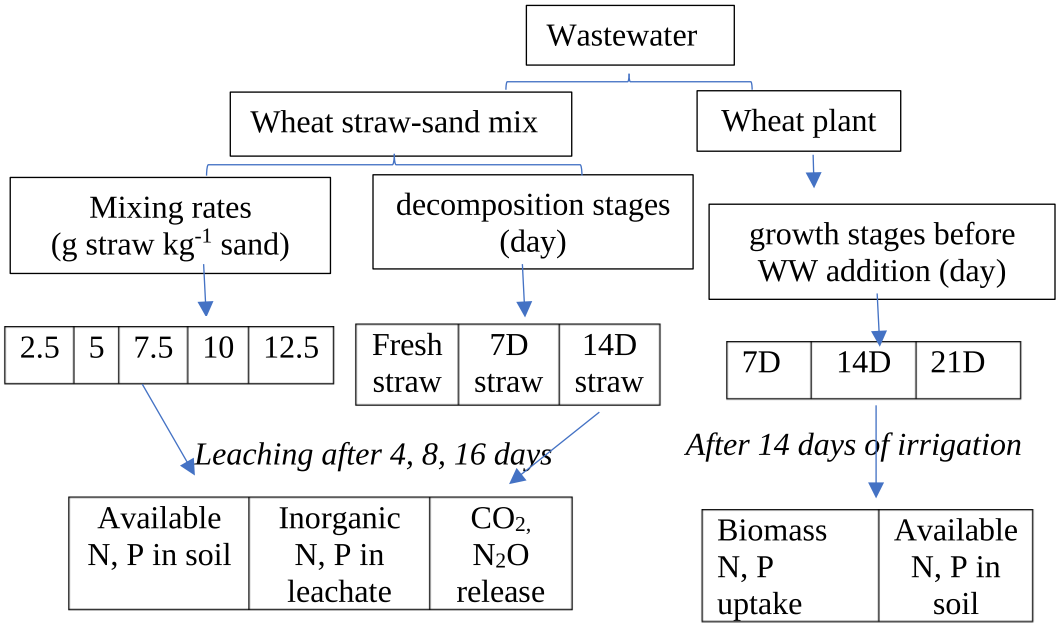

Figure 2. Design of the three main experiments in this study.

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CHAPTER 2

NITROGEN AND PHOSPHORUS REMOVAL FROM WASTEWATER BY SAND WITH WHEAT STRAW

Thi Huong Xuan Le[1, 3], Luke Mosley[2], Petra Marschner[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia.

[2]School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

[3]Hue University of Agriculture and Forestry, Hue City, Vietnam.

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Principal Author

Name of Principal Author (Candidate)	Thi Huong Xuan Le		
Contribute to the Paper	Performed experiment, analyses of soil and wastewater samples, data analysis and interpretation, wrote manuscript.		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	21 / 08/ 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

Name of Co-Author	Luke Mosley		
Contribution to the Paper	Contributed to planning of experiments, assisted in wastewater collection, discussions, proof-reading of manuscripts, supervised development of work		
Signature		Date	21/8/19

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, data interpretation, manuscript evaluation and correction and acted as corresponding author		
Signature		Date	21/08/2019

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RESEARCH ARTICLE

Nitrogen and phosphorus removal from wastewater by sand with wheat straw

Thi Huong Xuan Le[1,2] · Luke Mosley[3] · Petra Marschner[1]

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Abstract
Wheat straw amendment to sandy soil has the potential to remove nutrients from wastewater. This study investigated the ability of wheat straw to remove inorganic nitrogen (N) and phosphorus (P) from wastewater when mixed into sand at different rates. Wastewater from a sewage treatment plant was added to sand alone and amended with different wheat straw rates 2.5, 5, 7.5, 10, and 12.5 g wheat straw kg^{-1} so that the sand was covered with about 15 cm of wastewater. Leaching was carried out after 4, 8, and 16 days and inorganic N and P were analysed after leaching in both the leachate and sand, as well as N_2O and CO_2 release. In the amended sand, nitrate was about fourfold lower throughout the experiment compared to sand alone. Ammonium was twofold higher than sand alone at 12.5 g straw kg^{-1} throughout the experiment and on day 16 also at ≥ 5 g straw kg^{-1}. Leachate inorganic N concentration was up to 70-fold higher in sand alone than in amended soils irrespective of straw rate. On day 16, P leaching was about threefold lower and P retention was 40% higher in all amended treatments than sand alone. The redox potential in sand alone was higher than with straw amendments. With straw amendment, the release of CO_2 per day was six times higher than with sand alone and increased with straw rates, but very little N_2O and CH_4 was released throughout the experiment. It can be concluded that amendment of sand with wheat straw can remove large proportions of inorganic N and P from wastewater, even at low straw rates. Likely mechanisms for retention are dissimilatory nitrate reduction and subsequent binding of ammonium to straw for N, and binding to the straw and microbial uptake for P.

Keywords Available N · Available P · Leachate · Removal efficiency · Wastewater · Wheat straw

Introduction

Wastewater derived from anthropogenic activities is an environmental concern worldwide (Bedessem et al. 2005; Gibert et al. 2008). Nitrogen (N) enrichment in wastewater discharge can result in deterioration of aquatic ecosystems causing environmental hazards such as toxic algae (Howarth et al. 2002). Most prevalent forms of nitrogen in wastewater are ammonium (NH_4^+-N), nitrate (NO_3^--N), and organic nitrogen (Sedlak 1991; Sotirakou et al. 1999). Nitrate readily leached through the soil because it is a negatively charged ion, which is repelled from soil particles (Shirmohammadi et al. 1991). In contrast, the positively charged NH_4^+-N can be bound to negatively charged soil particles, such as clay (Jellali et al. 2011; Wang et al. 2015). In sandy soil, in particular, nitrate is leached more rapidly than in fine-textured soils because sandy soils have low water holding capacity, cation exchange capacity, and specific surface area for adsorption (Hamarashid et al. 2010). Hence, nitrate may contaminate groundwater, which, if the water is used as a drinking source, increases risk of cancer (Fewtrell 2004) and methemoglobinemia in infants (Ward et al. 2005). Several methods are used to remove nitrate and ammonium during wastewater treatment. In terms of nitrate removal, reverse osmosis ion exchange, electron dialysis, and activated carbon adsorption (Feleke and Sakakibara 2002; Islam and Suidan 1998; Schoeman 2009) have been applied presently. However, these methods have several disadvantages due to their cost and

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✉ Petra Marschner
petra.marschner@adelaide.edu.au

1 School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia

2 Hue University of Agriculture and Forestry, Hue City, Vietnam

3 School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

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generated by-products (Ghafari et al. 2008). Biological nitrate removal from wastewater has been utilised as a more economical alternative. Nitrate in wastewater can be converted to a variety of N forms by different biological pathways: (1) reduction to N_2 by heterotrophic bacteria under anaerobic conditions in biological denitrification (Ashok and Hait 2015; Warneke et al. 2011) where nitrate acts as the terminal electron acceptor for the oxidation of organic matter; (2) incorporation into algal or microbial biomass (Lance 1972; Lin and Stewart 1998); or (3) conversion to ammonium under anaerobic conditions in dissimilatory nitrate reduction to ammonium (DNRA) (Burgin and Hamilton 2007; Rezvani et al. 2017; Sander et al. 2015). According to Burgin and Hamilton (2007), biological denitrification and assimilation processes are assumed to be the main pathways for removal of nitrate in aquatic ecosystems. For controlling the amount of NH_4^+ in wastewater treatments, several methods such as chemical precipitation, ion exchange, supercritical water oxidation, microwave radiation, biological treatment, and adsorption techniques have been studied (Lin et al. 2009; Miladinovic and Weatherley 2008; Segond et al. 2002; Shi et al. 2013; Uludag-Demirer et al. 2005; Vassileva et al. 2009). Among these strategies, adsorption is considered a reliable and effective remediation which brings more economical advantages because it requires low energy input and easy operation (Ma et al. 2011).

Phosphorus is also one of the major nutrients accelerating eutrophication of lakes and natural water bodies, which is mostly derived from industrial and residential activities (Ruzhitskaya and Gogina 2017). The main forms of phosphorus compounds exist in wastewaters including orthophosphates, polyphosphates, and organic compounds (Sotirakou et al. 1999). Physical (e.g. adsorption and filtration), chemical (e.g. chemical precipitation and ion exchange), and biological (e.g. plant uptake and microbial degradation) techniques have been employed for P removal (Ma et al. 2011). Among these techniques, adsorption provides high efficiency in P removal (Wang et al. 2016b). However, the success of this technology depends on appropriate adsorbent (Onyango et al. 2007).

In biological wastewater treatment systems, various organic materials have been used as carbon substrates for microbial activity for example, woodchips (Christianson et al. 2017; Halaburka et al. 2017), biochar (Bock et al. 2016; Yang et al. 2018), and wheat straw (Aslan and Türkman 2004; Soares and Abeliovich 1998). Woodchips have been used for bio-filtration system to bind nutrients with removal of about 80% of total N and P added with wastewater (Choudhury et al. 2016; Ruane et al. 2011). Biochar has also been used as soil amendment after wastewater application, but its ability to retain nutrients depends on biochar properties (Wang et al. 2016a) and nutrient studied (Hale et al. 2013; Yao et al. 2012). Yao et al. (2012) tested 13 biochar types most of which sorbed little nitrate or phosphate. Only the biochar made from Brazilian pepperwood reduced nitrate, ammonium, and phosphate in the leachates by about 30% compared to soil alone. Wheat straw has been widely used as a low-cost material (Soares and Abeliovich 1998). For example, Fan et al. (2012) showed that wheat straw can be used as biofilm supporter for denitrification of synthetic wastewater with 100% nitrate removal within 15 days. Lowengart et al. (1993) suggested that when nitrogen-rich irrigation wastewater passed through a column of wheat straw with high C/N ratio, nitrogen immobilisation is expected to occur at the beginning, while a sequence of nitrification and denitrification would take place later. Although wheat straw has high capacity for nitrate removal from wastewater, the leachate may be coloured and the high dissolved organic carbon content in the effluent may make it unsuitable for drinking water (Aslan and Türkman 2004).

The ability of wheat straw on reducing the nutrient leaching in soils has been investigated; however, little is known about the influence of the application rate of wheat straw as an inexpensive soil amendment on nitrogen and phosphorus removal from wastewater. Many wastewater disposal systems, particularly in developing countries, are decentralised with direct application of untreated or poorly treated wastewater to soil. Further, there is increasing focus on recycling treated wastewater with final disposal on agricultural land. Improved strategies for minimising nutrient leaching would be valuable in order to reduce potential environmental impacts from these practices. However, it is also important to assess whether wheat straw amendment enhances greenhouse gas emissions (in particular nitrous oxide, N_2O, and carbon dioxide, CO_2) by enhancing microbial activity. If nutrients can be retained in wheat-straw-amended sand following wastewater application, this could open up new opportunities for agricultural production in previously infertile soils.

The aim of this study was to investigate the effect of different addition rates of wheat straw on inorganic N and P in leachate and nutrient availability in sandy soil following exposure to wastewater for different lengths of time. The following hypotheses were tested (i) wheat straw addition to sandy soil will increase N removal in applied wastewater compared to sand alone, (ii) N and P removal will increase with straw rate, and (iii) leachate composition and soil retention will change with incubation time. The second hypothesis is based on the assumption that with increasing rate, substrate surface area for adsorption and C supply for microbes will increase.

Materials and methods

Materials

Wastewater was collected from the Glenelg Sewage Treatment Plant in South Australia (longitude 138° 30′ 34.7″ E, latitude 34° 56′ 44.3″ S). Effluent for the experiments was collected after primary sedimentation and passage through active sludge bioreactors (Anonymous 2013). It was stored at 4 °C until use in the experiment. Nitrate-N, ammonium-N, and inorganic P concentrations in the wastewater (pH 7.0) were 7.9, 0.1, 4.9 mg L^{-1}, respectively.

Mature wheat straw (*Triticum aestivum* L.) was oven-dried at 40 °C, and ground and sieved to particle size from 0.25 to 2 mm before mixing into sand. It had the following properties: total organic C 391 g kg^{-1}, total N 5.5 g kg^{-1}, total P 1.7 g kg^{-1}, and C/N (71) and C/P ratio (228).

A coarse sand from a sand pit in South Australia was used (Santos Ready Mixed Concrete Pty Ltd). The sand was air-dried and sieved to particle size < 2 mm. It had the following properties: 98% sand, 1% silt, 1% clay, water holding capacity 0.008 g water g^{-1} soil, $EC_{1:5}$ 14.3 μS cm^{-1}, $pH_{1:5}$ 6.3, total organic C 0.18 g kg^{-1}, available N 11.93 mg kg^{-1}, and available P 0.36 mg kg^{-1}.

Experimental design

Wheat straw was thoroughly mixed into the sand at 2.5, 5, 7.5, 10, or 12.5 g dry weight kg^{-1} dry soil. The control was unamended sand. The sand alone or sand-wheat straw mixture (40 g) was placed in leaching columns constructed using 50-ml plastic syringes (3-cm diameter, 11-cm height). The leaching column outlet was closed with a three-way luer-lock valve. A plastic mesh (7.5 μm, Australian Filter Specialist) was placed on the bottom of the leaching column under the sand to prevent sand particles clogging the outlet. Before the addition of wastewater, both control and sand-wheat straw mixture were leached once with 25 ml RO water to remove any initial soluble nutrients in the sand and wheat straw. Then the bottom valve was closed, and 25 ml of wastewater was added. This volume of wastewater resulted in 1.3–1.5 cm height of wastewater above the sand surface and provided sufficient leachate for the analyses (22–25 ml). The leaching columns with wastewater were incubated upright at room temperature and leached after 4, 8, or 16 days with four replicates of each column. More than 85% of the applied wastewater leached within about 30 min, after which no further leaching occurred. Rapid leaching is common in sandy soils because they have mostly large pores that drain rapidly and therefore low water retention capacity. In this study, leaching was induced by opening the valve at the bottom of soil column, thus through gravity, as it would occur in the field. Before leaching, the leaching columns were placed in 1-L jars with gas-tight lids equipped with septa to quantify gas release. After 2 days, 10 ml of gas was removed from the headspace and injected into evacuated vials. Soil redox potential was measured immediately before leaching. Leaching was carried out by opening the bottom valve and collecting the leachate. Leachate ammonium and nitrate (inorganic nitrogen), inorganic P, and pH were measured. After leaching, soil was destructively sampled for determination of pH, available N, P, and microbial biomass N (MBN) and microbial biomass P (MBP).

Analyses

Available N in the sand-wheat mix was measured in a 1:5 soil:2 M KCl solution after 1-h end-over-end shaking. The extract was filtered before measuring the nitrate-N concentration after Miranda et al. (2001) at 540 nm and ammonium-N concentration after Willis et al. (1996) at 685 nm. Available P was extracted by the anion exchange resin method (Kouno et al. 1995) and measured at 712 nm.

Microbial biomass N (MBN) was measured by fumigation-extraction (Vance et al. 1987). Soil samples were fumigated with chloroform for 24 h followed by shaking with 0.5 mol L^{-1} K_2SO_4 at 1:4 soil extractant ratio for 1 h. MBN in extract was determined as ammonium-N as described above (Moore et al. 2001). MBN was calculated as the difference in ammonium-N concentration between fumigated and non-fumigated samples divided by 0.57 as suggested by Moore et al. (2000).

Microbial biomass P (MBP) was determined using the anion exchange method (Kouno et al. 1995). Hexanol 1 ml was used as fumigant. The P concentration was determined colorimetrically as described above for available P (Murphy and Riley 1962). MBP is the difference between fumigated and unfumigated samples. Soil pH was measured using a calibrated glass electrode in a 1:5 soil:water suspension.

Inorganic N and P of applied wastewater and in leachate were determined using the same colorimetric methods as for available N and available P. Redox potential was determined by using a platinum electrode connected to a smartCHEM-lab Multi-parameter Laboratory Analyser. ZoBell Redox standard solution was used for redox electrode calibration before every use. Prior to leaching, the redox electrode was inserted about 1 cm into the soil. Redox potential values (millivolts) were recorded after stabilisation. Recorded redox potential values were normalised to the potential of the standard hydrogen electrode by adding 240 mV to correct for the potential of the reference KCl electrode. Analysis of N_2O and CO_2 in head space gas was performed simultaneously using a Shimadzu GC-2014 Gas Chromatograph equipped with a Thermal Conductivity Detector and an Electron Capture Detector fitted for CO_2 and N_2O analysis, respectively. Helium was used as carrier gas.

The following calculations were carried out for total inorganic N and P in the leaching column (1), percentage of total N and P in the leaching column in the sand (2) and leachate (3), the percentage of inorganic N and P in applied wastewater as total inorganic N and P in leaching column (4), inorganic N and P in sand (5) and leachate (6) and removal efficiency (7):

$$Total\ inorganic\ N\ or\ P = inorganic\ N\ or\ P\ in\ sand + inorganic\ N\ or\ P\ in\ leachate \quad [\mu\text{g leaching column}^{-1}] \tag{1}$$

$$\%\ inorganic\ N\ or\ P\ in\ sand = \frac{inorganic\ N\ or\ P\ in\ sand}{total\ inorganic\ N\ or\ P} \times 100 \tag{2}$$

$$\%\ \text{inorganic N or P in leachate} = 100 - \%\ \text{inorganic N or P in sand} \tag{3}$$

$$\%^*\ \text{total inorganic N or P in leaching column} = \frac{total\ inorganic\ N\ or\ P}{inorganic\ N\ or\ P\ in\ added\ wastewater} \times 100 \tag{4}$$

$$\%^*\ \text{inorganic N or P in sand} = \frac{inorganic\ N\ or\ P\ in\ sand}{inorganic\ N\ or\ P\ in\ added\ wastewater} \times 100 \tag{5}$$

$$\%^*\ \text{inorganic N or P in leachate} = \frac{inorganic\ N\ or\ P\ in\ leachate}{inorganic\ N\ or\ P\ in\ added\ wastewater} \times 100 \tag{6}$$

(*): as percentage of inorganic N or P in applied wastewater

$$\text{RE}\ (\%) = \frac{(inorganic\ N\ or\ P\ in\ WW \times initial\ volume\ (25ml)) - (inorganic\ N\ or\ P\ in\ leachate \times LV)}{inorganic\ N\ or\ P\ in\ WW \times initial\ volume\ (25ml)} \times 100 \tag{7}$$

where RE, removal efficiency; WW, applied wastewater; LV, leachate volume.

Statistical analysis

The data of available N, P, MBN, and MBP in soil and leachate inorganic N and P was analysed by one-way repeated measures Analysis of Variance (ANOVA). Mean values at a given sampling time were compared using Tukey's multiple comparison tests at $P \leq 0.05$. Statistical analysis was carried out in IBM SPSS Statistics 24.

Results

Available N, P, microbial biomass, and redox potential in straw-sand mix

After leaching, nitrate in the straw-sand mix was three- to fourfold lower than that in sand alone (Fig. 1a). However, amendment ratio had no effect on nitrate concentration.

Ammonium in the straw-sand mix was higher than nitrate (Fig. 1b). Compared to sand alone, ammonium was twofold higher on days 4 and 8 only in the 12.5 g straw kg^{-1} amendment. But on day 16, it was two- to threefold higher in all amended treatments. In amended treatments on days 4 and 8, ammonium was about twofold higher with 12.5 g straw kg^{-1} amendment than with ≤ 7.5 g kg^{-1}. But on day 16, ammonium was similar in treatments with ≥ 5 g straw kg^{-1} which were 30–40% higher than 2.5 g straw kg^{-1}. With ≤ 7.5 g straw kg^{-1}, ammonium was about twofold higher on day 16 than on day 4. In the unamended sand and with 12.5 g straw kg^{-1}, ammonium was 30–40% higher on day 4 than on day 16.

Generally, inorganic N (sum of nitrate and ammonium, Fig. 1c) had similar treatment differences as ammonium. However, unlike ammonium, inorganic N in 12.5 g straw kg^{-1} was not different from sand alone on days 4 and 8.

Available P was not detectable on days 4 and 8 (Table S1). However, on day 16, available P was three to six times higher in sand alone than the amended treatments.

Redox potential before leaching (Fig. 1d) in sand alone was always positive (around + 300 mV) and much higher than in amended sand throughout the experiment. Redox potential was much lower (8–30 times) and changed over time in amended sand.

MBN ranged from 0.2 to 2.5 mg kg^{-1} and there were no significant differences among treatments (Table S1). MBP was not detectable on day 4. However, MBP ranged from 0.1 to 0.6 mg kg^{-1} on days 8 and 16. MBP did not differ between the treatments with ≤ 10 g straw kg^{-1}.

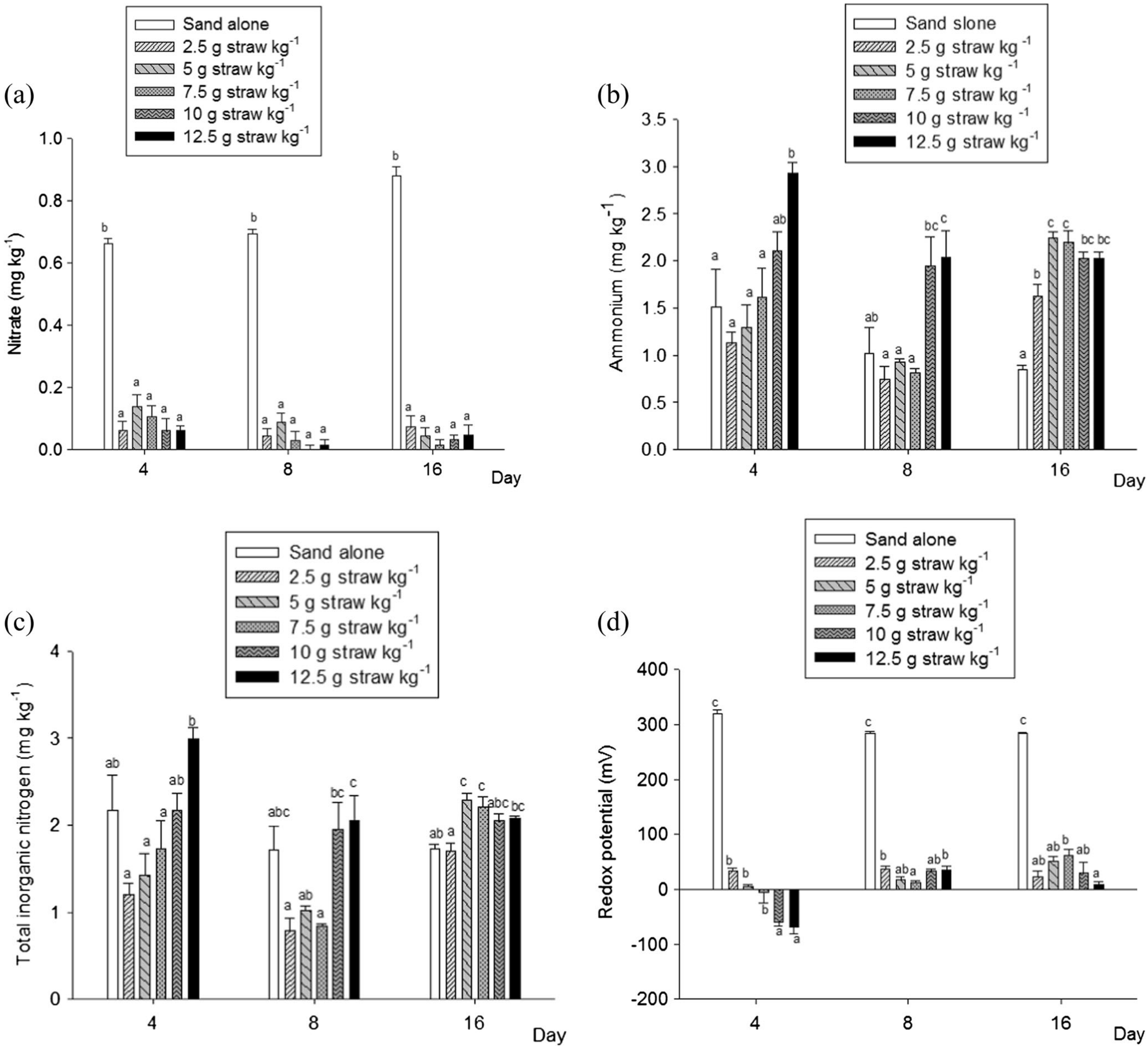

Fig. 1 Nitrate (**a**), ammonium (**b**), total inorganic nitrogen (**c**) in sand amended with 2.5–12.5 g straw kg^{-1} after leaching on days 4, 8, and 16, and redox potential (**d**) before leaching. At a given sampling time, columns with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Inorganic N and P in leachate

Leachate nitrate concentration (Table 1) in sand alone ranged from 6 to 7 mg L^{-1} which was at least 60 to 70-fold higher than that in amended sand. Straw addition rate had no effect on leachate nitrate. Leachate nitrate was higher on day 16 than on day 4 in all treatments.

Leachate ammonium (Fig. 2a) was lower than 0.5 mg L^{-1}. There was no significant difference between sand alone and amended sand except for a higher concentration in sand alone on day 8.

The pattern of leachate inorganic N (Fig. 2b) was similar to that of leachate nitrate. It was about 60- to 70-fold higher in sand alone than in amended soils. Amendment rate had no effect on inorganic N in leachate. Leachate inorganic N of sand alone was 17% higher on day 16 than on day 4.

Leachate inorganic P in sand alone was higher than in all amended treatments only on day 16 (Fig. 2c). For amended treatments, leachate inorganic P increased with straw rate. Compared to treatments with ≤ 5 g straw kg^{-1}, leachate inorganic P in 12.5 g straw kg^{-1} was threefold higher on days 4 and 8 and twofold higher on day 16. Leachate inorganic P decreased with incubation time, it was three to four times lower on day 16 than on day 4 in amended sand and two times lower in sand alone.

Soil pH ranged from 6.3 to 7. Leachate pH (Fig. 2d) in sand alone was higher than in all amended treatments on day 4, but only higher for ≥ 10 g straw kg^{-1} treatments on days 8 and 16.

Table 1 Leachate nitrate concentration in sand amended with 2.5–12.5 g straw kg^{-1} on days 4, 8, and 16. On a given day, values with different letters are significantly different ($n = 4$, $P \leq 0.05$). n.d. refers to not detectable which is < 0.1 mg L^{-1}

Treatment	Nitrate in leachate		
	mg L^{-1}		
	Day 4	Day 8	Day 16
Sand alone	6.0	6.1	7.1b
2.5 g straw kg^{-1}	n.d.	n.d.	0.0a
5.0 g straw kg^{-1}	n.d.	n.d.	0.1a
7.5 g straw kg^{-1}	n.d.	n.d.	0.1a
10.0 g straw kg^{-1}	n.d.	0.1	0.1a
12.5 g straw kg^{-1}	n.d.	0.1	0.3a

Total inorganic N and P in leaching column and the proportion of N and P in soil and leachate

Total inorganic N in leaching column in sand alone was two- to sixfold higher than in amended sand for all leaching events (Table 2). With 12.5 g straw kg^{-1}, total inorganic N was two- to threefold higher than with ≤ 7.5 g straw kg^{-1} on days 4 and 8. Over 90% of inorganic N was retained in straw-amended sand, only ≤ 9% of inorganic N was leached. With ≤ 7.5 g straw kg^{-1}, inorganic N in leaching columns was 30–40% higher on day 16 than on day 4, while it decreased by 7–30% with ≥ 10 g straw kg^{-1}. For sand alone, total inorganic N in the leaching column was stable throughout the experiment.

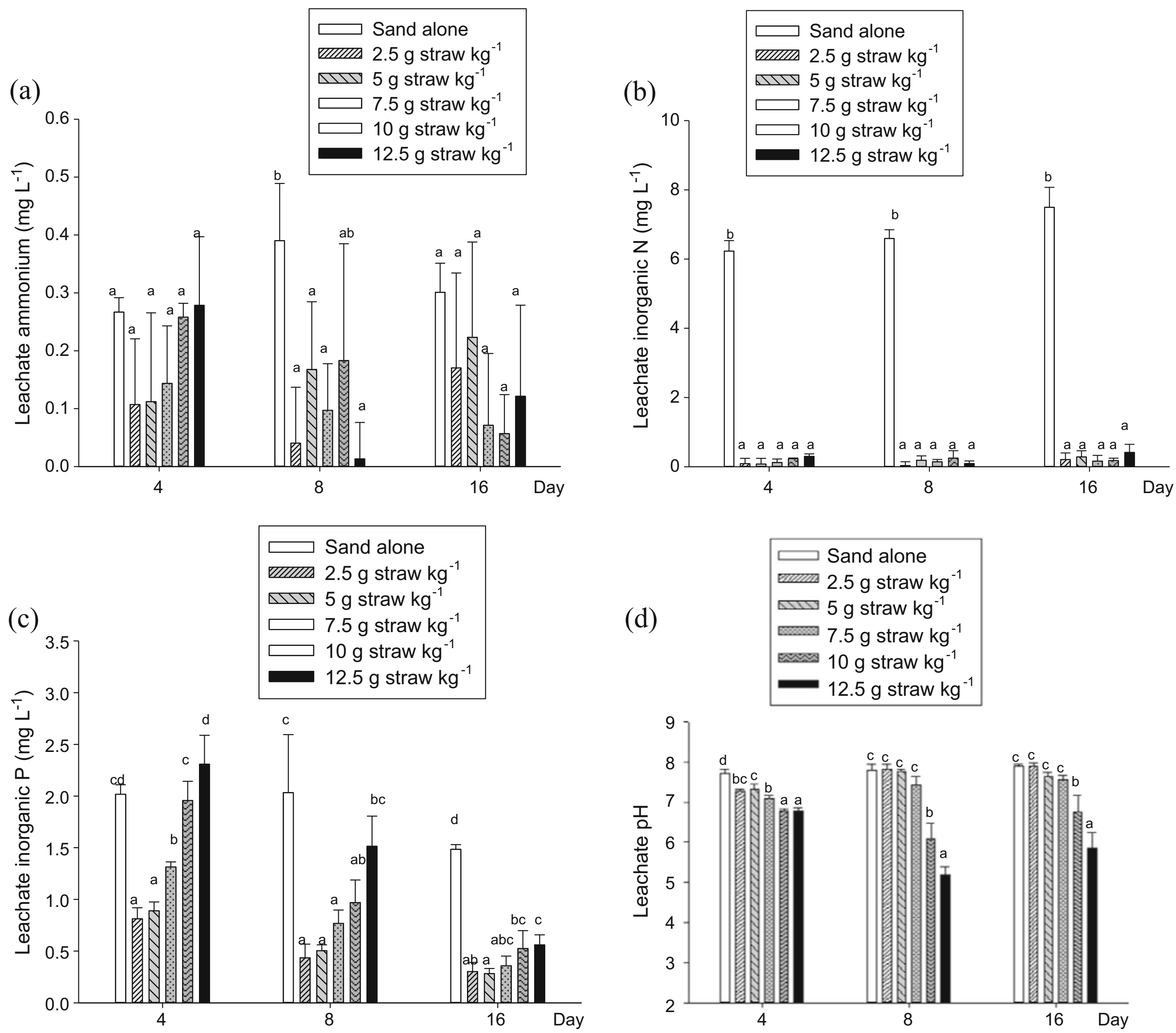

Fig. 2 Leachate ammonium (**a**), inorganic nitrogen (**b**), inorganic phosphorus (**c**), and pH (**d**) in sand amended with 2.5–12.5 g straw kg^{-1} on days 4, 8, and 16. At a given sampling time, columns with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Table 2 Inorganic N per leaching column as sum of inorganic N in sand amended with 2.5–12.5 g straw kg^{-1} and leachate and percentage of N in soil and leachate on days 4, 8, and 16. On a given day, values with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Treatment	Total inorganic N			% inorganic N in sand			% inorganic N in leachate		
	μg $column^{-1}$			%					
	Day 4	Day 8	Day 16	Day 4	Day 8	Da 16	Day 4	Day 8	Day 16
Sand alone	225c	209d	217c	38a	32a	32a	62b	68b	68b
2.5 g straw kg^{-1}	50a	33a	72a	96b	94b	94b	4a	6a	6a
5.0 g straw kg^{-1}	60a	45ab	97b	95b	91b	94b	5a	9a	6a
7.5 g straw kg^{-1}	72a	37a	92ab	95b	92b	97b	5a	9a	3a
10.0 g straw kg^{-1}	92ab	82bc	86ab	94b	95b	96b	6a	5a	4a
12.5 g straw kg^{-1}	127b	84c	91ab	94b	98b	91b	6a	2a	9a

Total inorganic P in the leaching column with sand alone was four- to sevenfold higher than in amended treatments only on day 16, it was lower or similar to 12.5 g straw kg^{-1} on days 4 and 8 (Table 3). Total inorganic P was about two- to threefold higher on day 4 than on day 16 in amended sand, but it stayed the same in sand alone. In amended sand, total inorganic P increased with straw rate on days 4 and 8, but it was not influenced by rate on day 16. Unlike total inorganic N, the majority of inorganic P (70–100%) was found in leachates.

Release of N_2O, CO_2, and CH_4

Release of N_2O per day was low (< 0.25 ppm) in all treatments (Fig. 3a). For amended sand, released N_2O was about 20% higher than in sand alone before the day 4 leaching. But later, it was similar or lower than in sand alone. For amended sand, released N_2O was about 30% lower before leaching on day 16 than before day 4. Released N_2O per day (data not shown) was less than 1% of total inorganic N in leaching column.

Release of CO_2 (Fig. 3b) in sand alone was about two to six times lower than in amended sand for all leaching events. Released CO_2 was 15–70% higher in 12.5 g straw kg^{-1} than in ≤ 7.5 g straw kg^{-1}. With ≥ 7.5 g straw kg^{-1}, released CO_2 before the day 16 sampling was 30–40% higher than before the day 4 sampling. However, released CO_2 in sand alone and with 2.5 g straw kg^{-1} was stable throughout the experiment.

Released CH_4 per day (Figure 1S) was low (< 1 ppm) and variable throughout the experiment. CH_4 release did not differ among treatments before leaching on day 4 and 8. However on day 16, released CH_4 per day with ≥10 g straw kg^{-1} was 35% higher than with 2.5 g straw kg^{-1}.

Total inorganic N and P in leaching column, the inorganic N and P in soil, and leachate as percentage of inorganic N and P in the added wastewater

As percentage of inorganic N in the added wastewater, total inorganic N in leaching column with sand alone was two- to sixfold higher than with amended sand (Table 4). For amended treatments, the percentage of inorganic N in the added wastewater in leaching columns with 12.5 g straw kg^{-1} was two times higher than with ≤ 7.5 g straw kg^{-1} only on days 4 and 8. The percentage of inorganic N in soil was two to five times lower than inorganic N in added wastewater for all treatments. It was similar in all treatments on days 4 and 8. With 12.5 g straw kg^{-1}, it was two times higher than with ≤ 7.5 g straw kg^{-1} on days 4 and 8, but was similar with ≥ 5 g

Table 3 Inorganic P per leaching column as sum of inorganic P in sand amended with 2.5–12.5 g straw kg^{-1} and leachate percentage of P in soil and leachate on days 4, 8, and 16. On a given day, values with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Treatment	Total inorganic P			% inorganic P in sand			% inorganic P in leachate		
	μg $column^{-1}$			%					
	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16	Day 4	Day 8	Day16
Sand alone	45c	50c	53b	0a	12b	45a	100a	87a	55a
2.5 g straw kg^{-1}	19a	10a	11a	0a	0a	29a	100a	100b	71a
5.0 g straw kg^{-1}	21a	11a	8a	0a	0a	24a	100a	100b	76a
7.5 g straw kg^{-1}	32 b	17ab	11a	0a	0a	27a	100a	100b	74a
10.0 g straw kg^{-1}	46c	20ab	17a	0a	0a	33a	100a	100b	67a
12.5 g straw kg^{-1}	58d	33bc	14a	0a	0a	16a	100a	100b	84a

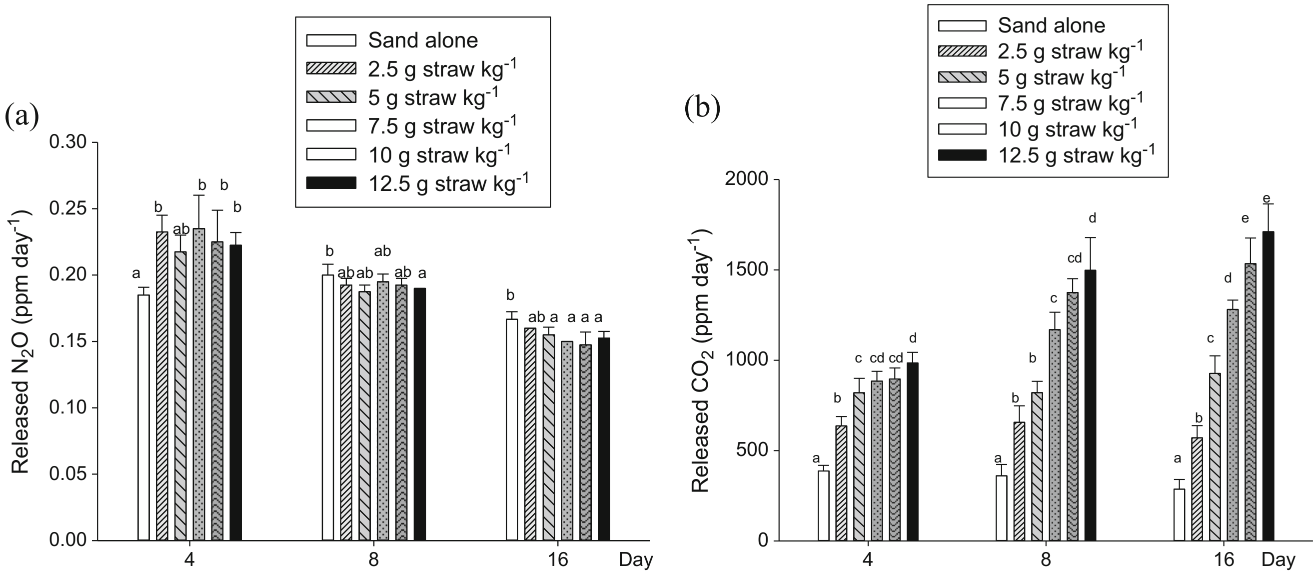

Fig. 3 Released N_2O (**a**) and CO_2 (**b**) in ppm day^{-1} from sand amended with 2.5–12.5 g straw kg^{-1} in the 2 days prior to leaching on days 4, 8, and 16. At a given sampling time, columns with different letters are significantly different ($n = 4$, $P \leq 0.05$)

straw kg^{-1} on day 16. The percentage of inorganic N in leachate with sand alone was 70% higher than in all amended treatments for all leaching events. With ≤ 7.5 g straw kg^{-1}, the percentage of inorganic N in leaching columns was 20–30% higher on day 16 than day 4, while it decreased by 9–40% with ≥ 10 g straw kg^{-1}. For sand alone, the percentage of total inorganic N in the leaching column was stable throughout the experiment.

The percentage of total inorganic P in the leaching column with sand alone was lower or similar to 12.5 g straw kg^{-1} on days 4 and 8 (Table 5). Only on day 16, it was four- to sevenfold higher than in amended treatments. In amended sand, the percentage of total inorganic P in the leaching column increased with straw rate on days 4 and 8, but was not influenced by rate on day 16. Only on day 16, the percentage of inorganic P in leachate with sand alone was only higher than with 12.5 g straw kg^{-1}. The percentage of inorganic P in leachate increased with straw rate. But straw rate had no effect on the percentage of inorganic P in added wastewater as inorganic P in soil which was undetectable on day 4 in all treatments. In amended treatments, it was also undetectable on day 8 and > 5% on day 16. In amended sand, the percentage of total inorganic P in soil changed little over time, but with sand alone it was fourfold higher on day 16 than on day 8.

N and P removal efficiency

In amended sand, 96–100% of applied N was removed irrespective of straw rate. In sand alone, only 25–30% was removed (Table 6). In sand alone, 64–76% of applied P was removed compared to 53–96% in amended treatments (Table 6). In amended treatments, removal was greater with 2.5 g straw kg^{-1} than with 12.5 g straw kg^{-1}. In all treatments, P removal was 11–38% greater on day 16 than day 4.

Table 4 Total inorganic N (sum of inorganic N in sand amended with 2.5–12.5 g straw kg^{-1} and leachate), inorganic N in sand and leachate on days 4, 8, and 16 as percentage of inorganic N in applied wastewater. On a given day, values with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Treatment	Total inorganic N			Inorganic N in sand			Inorganic N in leachate		
	As percentage of inorganic N added (%)								
	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16
Sand alone	114c	106d	110c	44ab	35abc	35ab	70b	71b	75b
2.5 g straw kg^{-1}	26a	17a	37a	24a	16a	34a	1a	1a	2a
5.0 g straw kg^{-1}	30a	23ab	49b	29a	21ab	46c	1a	2a	3a
7.5 g straw kg^{-1}	36a	19a	46ab	35a	17a	45c	1a	2a	2a
10.0 g straw kg^{-1}	47ab	42bc	43ab	44ab	39bc	41abc	3a	2a	2a
12.5 g straw kg^{-1}	64b	43c	46ab	60b	42c	42bc	4a	1a	4a

Table 5 Total inorganic P (sum of inorganic P in sand amended with 2.5–12.5 g straw kg^{-1} and leachate), inorganic P in sand and leachate on days 4, 8, and 16 as percentage of inorganic P in applied wastewater. On a given day, values with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Treatment	Total inorganic P			Inorganic P in sand			Inorganic P in leachate		
	As percentage of inorganic P added (%)								
	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16
Sand alone	36c	40c	43b	n.d	5	19b	36c	35c	24d
2.5 g straw kg^{-1}	15a	8a	9a	n.d	n.d	4a	15a	8a	5ab
5.0 g straw kg^{-1}	17a	9a	6a	n.d	n.d	2a	17a	9a	4a
7.5 g straw kg^{-1}	26b	14ab	9a	n.d	n.d	3a	26b	14ab	6abc
10.0 g straw kg^{-1}	38c	16cb	13a	n.d	n.d	5a	38c	16ab	8bc
12.5 g straw kg^{-1}	47d	27bc	11a	n.d	n.d	2a	47d	27bc	9c

Discussion

Based on this study, the first hypotheses (wheat straw addition to sand will increase N removal in applied wastewater compared to sand alone) can be accepted, but the second hypothesis (N and P removal will increase with straw rate) has to be declined. The third hypothesis (leachate composition and soil retention will change with incubation time) can be confirmed.

In sand alone, where only the wastewater would supply organic C in addition to the small amount in the sand, redox potential remained high which can be explained by the low substrate availability to microbes (Lescure et al. 1992) and therefore low O_2 consumption and CO_2 release (Tokarz and Urban 2015). Nitrate concentrations in soil and leachate were higher than in amended soils whereas ammonium concentrations were lower. This suggests that there was little dissimilatory nitrate reduction to ammonium in the unamended sand (Table 7), which can also be explained by the low organic C availability (Liu et al. 2016).

In amended treatments, CO_2 release was higher than sand alone particularly at high straw rates due to the supply of organic C by straw to microbes (Reinertsen et al. 1984) which resulted in consumption of O_2 and other electron acceptors (e.g. nitrate), and consequently reduced the redox potential compared to sand alone. Compared to sand alone, nitrate concentrations in soil and leachate were much lower and soil ammonium concentrations were higher which can be explained by dissimilatory nitrate reduction to ammonium as a result of the supply of organic C by straw (Liu et al. 2016). On day 4, available ammonium was highest at 12.5 g kg^{-1}, likely because it supplied the greatest amount of C, but on day 16, available ammonium was similar in amended treatments, indicating that over time as straw is decomposed, even low straw rates are sufficient to stimulate dissimilatory nitrate reduction to ammonium (Table 7).

Table 6 Inorganic N and P removed in percentage of amounts in applied wastewater. On a given day, values with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Treatment	Inorganic N removal			Inorganic P removal		
	(%)					
	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16
Sand alone	30a	29.0a	25a	64b	65a	76a
2.5 g straw kg^{-1}	99b	100b	98b	84d	92c	95cd
5.0 g straw kg^{-1}	99b	98b	97b	83d	91c	96d
7.5 g straw kg^{-1}	99b	98b	98b	74c	86c	94bcd
10.0 g straw kg^{-1}	97b	97b	98b	62b	84bc	92bc
12.5 g straw kg^{-1}	96b	99b	96b	53a	74ab	91b

Nitrate is easily leached because of its negative charge (Shirmohammadi et al. 1991). The positively charged ammonium on the other hand can be bound to negatively charged sites, e.g. soil exchange sites and on organic materials (Jellali et al. 2011; Wang et al. 2015). Wheat straw contains cellulose (32%), hemicellulose (29%), and lignin (16%) (Gao et al. 2016). Dang et al. (2015) found that wheat straw had a cation exchange capacity of 15.6 $cmol_c$ kg^{-1} and a surface area of 0.8 m^2 g^{-1} which were lower than those of biochar. The main forms of organic C in wheat straw were (in percentage organic C detected) alkyl 4.8, N-alkyl/methoxyl 4.3, O-alkyl 61.3, di-O-alkyl 14.1, aryl 7.4, O-aryl 3.2, and amide/carboxyl 4.1. O/N alkyl C groups that can bind polar compounds such as NH_4^+ and other cations (Jassal et al. 2015). This may explain why although available ammonium on days 4 and 8 was highest with 12.5 g straw kg^{-1}, leached ammonium concentrations were similar in all treatments. With the highest straw rate, there are likely to be more potential ammonium binding sites than with low rates. In the unamended sand on the other hand, little ammonium was produced and correspondingly leachate ammonium concentrations did not differ from those in amended soils.

Throughout the experiment, N_2O release differed little between treatments and was low suggesting that denitrification was not an important process in this experiment. The small amounts of N_2O released may be a by-product of dissimilatory nitrate reduction (Kraft et al. 2011).

Table 7 Fate of nitrate in wastewater added to sand alone or straw-sand mixes

Treatment	Organic C supply	O_2 consumption and CO_2 release	RP*	NO_3^- in WW**	N2O release	DN***	NO_3^- concentration in soil and leachate	NH_4^+ concentration in soil	DNRA	NH_4^+ binding to sand/straw-sand mixes
Sand alone	Low	Low	High	Acts as electron acceptor	Very low	Very low	High	Low	Low	Low
Wheat straw 2.5–12.5 g kg^{-1}	High	High	Low		Very low	Very low	Low	High	High	High

*RP redox potential; **wastewater; ***denitrification

Straw rates < 10 g kg^{-1} reduced P leaching compared to sand alone throughout the experiment which can be due to P binding to the straw (Ma et al. 2011), and on day 16 uptake into to the microbial biomass. At higher straw rates, P leaching was only lower than in sand alone on day 16. The initial lack of reduction in P leaching at these higher rates could be due to organic acid anions produced during decomposition of the high straw rates which can be bound to the same sorption sites as P and therefore inhibit P binding (Iyamuremye et al. 1996; Jalali 2009). After 16 days, leachate P concentration was low in all amended treatments, probably because even at the highest straw rate, less organic acid anions were produced, and more P was taken up by the microbial biomass.

Inorganic N in leachate in amended treatments as percentage of inorganic N in the added wastewater was much lower than in sand alone. This is likely because the main N form leached was nitrate and nitrate leaching was extremely low in all straw amendments due to dissimilatory nitrate reduction. As percentage of inorganic P in the added wastewater, inorganic P in leachate of amended treatments reduced overtime and they all were lower than in sand alone only on day 16. This is because more P was bound to straw—sand mixes and microbial biomass uptake over time.

Conclusion

This study showed that wheat straw amendment to sand has the potential to reduce leaching of inorganic N and P. Even at low addition rates, a much large proportion of inorganic N applied with wastewater was retained than in sand alone. Low straw rates were also more effective to remove inorganic P compared to high rates. Nutrients retained in amended sandy soils could be potentially available for plant uptake a valuable nutrient source for crops. Further studies are required to investigate the longer term effect of wheat straw which may be different from this short-term study due to decomposition of the straw. This method would also need to be tested at the field-scale to assess its applicability.

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CHAPTER 3

WHEAT STRAW DECOMPOSITION STAGE HAS LITTLE EFFECT ON THE REMOVAL OF INORGANIC N AND P FROM WASTEWATER LEACHED THROUGH SAND-STRAW MIXES

Thi Huong Xuan Le[1, 3], Luke Mosley[2], Petra Marschner[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia.

[2]School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

[3]Hue University of Agriculture and Forestry, Hue City, Vietnam.

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Principal Author

Name of Principal Author (Candidate)	Thi Huong Xuan Le		
Contribute to the Paper	Performed experiment, analyses of soil and wastewater samples, data analysis and interpretation, wrote manuscript and acted as corresponding author.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

Name of Co-Author	Luke Mosley		
Contribution to the Paper	Contributed to planning of experiments, assisted in wastewater collection, discussions, proof-reading of manuscripts, supervised development of work		
Signature		Date	21/8/19

Name of Co-Author	Petra Marschner		
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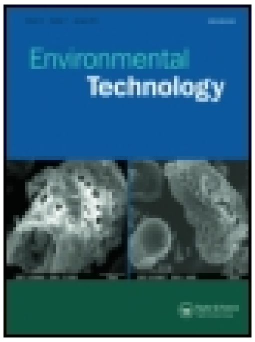

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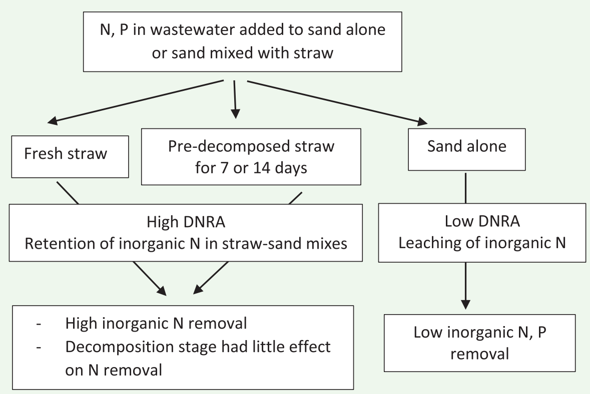
Thi Huong Xuan Le[a,b], Luke Mosley[c] and Petra Marschner[a]

[a]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, Australia; [b]Hue University of Agriculture and Forestry, Hue City, Vietnam; [c]School of Biological Sciences, The University of Adelaide, Adelaide, Australia

ABSTRACT

Wheat straw amendment to sandy soil can remove nitrogen (N) and phosphorus (P) from wastewater but it is unclear whether prior decomposition affects removal. Sand mixed with finely ground wheat straw at 12.5 g straw kg^{-1} was placed in leaching columns. Wastewater was added either immediately after mixing with straw (fresh straw) or after the sand-straw mix had been incubated moist for 7 or 14 days (7D or 14D straw). Sand alone was considered as control. Leaching was carried out 4, 8 or 16 days after addition of wastewater and inorganic N and P were analysed after leaching in both leachate and sand. In the amended treatments, nitrate and available P in the sand-straw mix were not detectable throughout the experiment. On day 16, inorganic N in the sand-straw mix was highest in fresh straw where it was three-fold higher than in 14D straw and 30% higher than in sand alone and 7D straw on day 16. Straw decomposition stage had no consistent effect on microbial biomass N and P. Released CO_2 was lower in 14D straw than in fresh straw and 7D straw. With straw amendment, > 95% of inorganic N added with wastewater was removed compared to 40–50% with sand alone. Inorganic P leaching was reduced by about 30% compared to sand alone on day 16. In conclusion, wheat straw addition reduced leaching of N compared to sand alone, but the decomposition stage of the straw had little effect on the removal of N and P from wastewater.



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Available N; available P; leachate; wastewater; wheat straw

1. Introduction

Increased nitrogen (N) and phosphorus (P) inputs have resulted in accelerated water eutrophication worldwide [1,2]. Inorganic N enters water ways mainly as nitrate because in soils, the negatively charged NO_3^-N is repelled [3], whereas the positively charged NH_4^+-N can be bound to negatively charged soil particles, such as clay [4,5]. Excessive nitrate inputs to soil may contaminate both groundwater and drinking water and increase the risk of cancer [6] and methemoglobinemia in infants [7]. Further, high concentrations of dissolved N and P in domestic and industrial wastewater can result in environmental and aesthetic problems in water bodies such as excessive growth of blue green algae and plants [1]. Wastewater application to sandy soils is particularly problematic because sandy soils have high nutrient leaching potential due to their low water holding capacity, low-specific surface area for adsorption [8], and low cation exchange capacity. Research is needed to inform the development of management strategies to minimize nutrient leaching.

CONTACT Thi Huong Xuan Le thi.h.le@adelaide.edu.au School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia; Hue University of Agriculture and Forestry, Hue City, Vietnam
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Physical, chemical, physio-chemical and biological technologies are used to remove nitrate [9,10] and P [11] in wastewater treatment plants. Among them, biological removal is considered as the most economical method [12]. Nitrate in wastewater can be transformed to different N forms by various biological pathways: (1) it can be incorporated into algal or microbial biomass [13,14]; in anaerobic conditions (2) it can be reduced to N_2O by heterotrophic bacteria in biological denitrification [15,16] where nitrate acts as the terminal electron acceptor for the oxidation of organic matter or; (3) it can be converted to ammonium in dissimilatory nitrate reduction to ammonium (DNRA) [17–19]. In biological wastewater treatment systems, organic materials are often used as carbon substrates for microbes to enhance nutrient removal from wastewater [9,20]. Organic materials can also be used as soil amendment to minimize contamination of ground and surface water. For example, woodchips [21], biochar [22] and wheat straw [20] amendment has been shown to reduce nutrient leaching from soils receiving wastewater. Woodchips have been used as a bio-filtration system to absorb nutrients in wastewater with the removal efficiency about 80% of total N and P of the influent [23,24]. Biochar has a high capacity for mineral sorption and has been used as amendment for sandy soil to regulate nutrient bioavailability in soil [25] and leachate composition [26]. However, its ability to retain nutrients depends on biochar properties [27] and nutrient types [26,28]. Compared to other agricultural residues, wheat straw is relatively low cost and widely available [9,29]. Cellulose, hemicelluloses and lignin (32, 29, and 16% of total organic C) in wheat straw have a large number of functional groups [30] that can bind polar compounds such as NH_4^+ and other cations [31]. For example, wheat straw was used to remove metal ions in wastewater [32,33]. Wheat straw has also been previously used to bind ammonium-N from cattle urine [34] and as a biofilm supporter for denitrification of synthetic wastewater under anaerobic conditions [35].

Straw incorporation into soil, e.g. after crop harvest, can improve aggregate stability in the field [36]. Straw could also be added before wastewater irrigation. However, it is unclear if decomposition of wheat straw soil amendments before application of wastewater influences its capacity to remove N and P. Decomposition of wheat straw leads to several changes in chemical properties. In the initial stages of decomposition, water-soluble components are rapidly depleted resulting in high respiration rates [37]. Later, hemicellulose and cellulose and other complex compounds are mineralized [38,39]. During the first two weeks of straw decomposition, high proportions of organic acid anions are released [40]. These organic acids can influence nutrient availability by competing for binding sites on soil particle surfaces [41]. In addition, C-containing functional groups change during wheat straw decomposition, particularly O-alkyl C to alkyl C and aromatic to COO/N–C=O groups [30]. These changes in functional groups could affect binding of cationic and anionic nutrients. Decomposition of wheat straw can also induce nutrient immobilization by the microbial biomass. When N-rich wastewater was passed through a column packed with wheat straw (C/N 112), net nitrogen immobilization occurred at the beginning, while later nitrification and denitrification dominated [42].

Using sand mixed with wheat straw, our study aims to investigate the influence of decomposition stage of the straw and the duration of contact with wastewater on inorganic N and P in leachate and the sand-straw mix. The hypotheses were that (i) N and P leaching will be lower with straw than in unamended sand, (ii) removal will be greater with fresh straw than with pre-decomposed straw, and (iii) leachate composition and soil retention will change with incubation time. The second hypothesis is based on the assumption that compared to fresh straw, prior decomposition of wheat straw changes its functional groups as well as availability to microbes thereby reducing N and P binding by straw and microbial N and P uptake.

2. Materials and methods

2.1. Materials

Wastewater was collected from the Glenelg Sewage Treatment Plant in South Australia (Longitude 138° 30′34.7″E Latitude 34°56′44.3″S). Effluent for the experiments was collected after primary sedimentation and passage through active sludge bioreactors [43]. Nitrate, ammonium and inorganic P concentrations in the wastewater (pH 7.0) were 7.9, 0.1, 4.9 mg l^{-1}, respectively.

A coarse sand from a sand pit in South Australia was used (Santos Ready Mixed Concrete Pty Ltd). The sand was air-dried and sieved to particle size < 2 mm. It had the following properties: 98% sand, 1% silt, 1% clay, water holding capacity 0.008 g water g^{-1} soil, $EC_{1:5}$ 14.3 µS cm^{-1} and $pH_{1:5}$ 6.3, total organic C 0.18 g kg^{-1}, available N 11.93 mg kg^{-1} and available P 0.36 mg kg^{-1}.

Mature wheat straw (*Triticum aestivum* L.) was oven-dried at 40°C, ground and sieved to particle size 0.25–2 mm before mixing into sand. It had the following properties: total C 391 g kg^{-1}, total N 5.5 g kg^{-1}, total P 1.7 g kg^{-1}; C/N 71 and C/P ratio 228.

2.2. Experimental design

The leaching columns were 50 ml plastic syringes (3 cm diameter, 11 cm height) without the plunger. The leaching column outlet faced down and was closed with a luer-lock valve. A plastic mesh (7.5 µm, Australian Filter Specialist) was placed on the bottom of the column to prevent sand particles clogging the outlet. Wheat straw was thoroughly mixed into the sand at 12.5 g dry weight kg^{-1} dry soil. A previous study had shown that this straw rate had high efficiency in N and P removal from wastewater [44]. Sand alone or sand-wheat straw mixture (40 g) was placed in the leaching columns. Wastewater was added either immediately after mixing of sand and straw or after incubation at 80% WHC at room temperature for 7 or 14 days, treatments are referred to as fresh straw, 7D straw and 14D straw, respectively. The incubation times were chosen based on Nguyen et al. [45], who found that decomposition rates of wheat straw are high in the first 2–3 days, then decrease slowly until about day 7 after which they are low and stable. Therefore, we assumed that easily decomposable compounds are depleted after 7 days, followed by more slow decomposition of more complex compounds such as cellulose and lignin. The control was unamended sand. Wastewater was added at 25 ml/leaching column which resulted in a height of 1.3 cm of water above the sand surface and was enough to obtain sufficient leachate for the analyses (12–15 ml). The columns with wastewater were incubated at room temperature and leached after 4, 8 or 16 days with four replicates of each treatment. Before leaching, the columns were placed in 1 L jars with gas-tight lids equipped with septa to quantify the gas. After 2 days, 10 ml of gas was removed from the headspace and injected into evacuated vials. Soil redox potential was measured one day before leaching. Leachate NH_4^+-N and NO_3^-N, inorganic P and pH were measured. After leaching, soil was destructively sampled for determination of pH, available N, P and microbial biomass N (MBN) and P (MBP).

2.3. Analyses

Soil texture was determined by the hydrometer method [46]. Soil pH was measured in a 1:5 soil:water suspension using a calibrated glass electrode after 1 h end-over-end shaking at 25°C [47]. Water holding capacity of the sand and sand-straw mixes was measured using a sintered glass funnel connected to a 1 m water column (matric potential = −10 kPa) [48].

Redox potential was determined before leaching using a platinum electrode connected to a smartCHEM-lab Multi-parameter Laboratory Analyser. The redox electrode was inserted into the soil about 1 cm. Redox potential values (millivolts) were recorded after stabilization. ZoBell's Redox standard solution was used for calibration before every use. Recorded redox potential values were normalized by adding 240 mV to correct for the potential of the reference electrode.

Nutrient and microbial biomass analyses were carried out as described in Marschner et al. [49]. Available N (nitrate + ammonium) concentration was measured in a 1:5 soil 2 M KCl extract after 1 h end-over-end shaking. The extract was used to colorimetrically measure the nitrate-N concentration at 540 nm as demonstrated by Miranda et al. [50] and ammonium-N concentration at 685 nm as demonstrated by Willis et al. [51]. Detection limits for nitrate and ammonium were 0.07 and 0.1 mg l^{-1}, respectively. Available P concentration was determined colorimetrically at 712 nm [52] after being extracted by the anion exchange resin method [53]. Detection limit for P was 0.05 mg l^{-1}.

Inorganic N, P of applied wastewater and in leachate were determined using the same analytical methods as for available N and P after extraction.

Microbial biomass nitrogen (MBN) was determined by chloroform fumigation-extraction with 0.5 M K_2SO_4 at 1:4 soil to extractant ratio [54]. MBN in the extract was measured colorimetrically by the same method as ammonium-N determination [55]. MBN was calculated as the difference in ammonium-N concentration between fumigated and non-fumigated samples divided by 0.57 as suggested by Moore et al. [54]. Microbial biomass P (MBP) was determined using the anion exchange method [53] using hexanol as a fumigant. P concentration in the extract was measured colorimetrically as described above for available P [52]. MBP is the difference between fumigated and unfumigated samples.

N_2O, CO_2, and CH_4 were measured simultaneously using a Shimadzu GC-2014 Gas Chromatograph equipped with a Flame-Ionization Detector, Thermal Conductivity Detector, and an Electron Capture Detector fitted for CH_4, CO_2 and N_2O analysis, respectively. Helium was used as a carrier gas.

The following calculations were carried out for total inorganic N and P in the leaching column (1), percentage of total N and P in the leaching column in the sand/sand-wheat mixes (2) and leachate, (3) percentage of inorganic N and P in applied wastewater as total inorganic N and P in leaching column (4), inorganic N and P in sand (5) and leachate (6):

$$\text{Total inorganic N or P} = \text{inorganic N or P in sand} + \text{inorganic N or P in leachate} \quad (1)$$

in [µg leaching column^{-1}]

$$\%\text{inorganic N or P in sand} = \frac{\text{inorganic N or P in sand}}{\text{total inorganic N or P}} \times 100 \quad (2)$$

$$\%\ \text{inorganic N or P in leachate} = 100 - \%\ \text{inorganic N or P in sand} \quad (3)$$

$$\%{*}\ \text{total inorganic N or P in leaching column} = \frac{\text{total inorganic N or P}}{\text{inorganic N or P in added wastewater}} \times 100 \quad (4)$$

$$\%{*}\ \text{inorganic N or P in sand} = \frac{\text{inorganic N or P in sand}}{\text{inorganic N or P in added wastewater}} \times 100 \quad (5)$$

$$\%{*}\ \text{inorganic N or P in leachate} = \frac{\text{inorganic N or P in leachate}}{\text{inorganic N or P in added wastewater}} \times 100 \quad (6)$$

*: as percentage of inorganic N or P in applied wastewater

2.4. Statistical analysis

The data of available N, P, MBN and MBP in soil and leachate inorganic N and P were analysed by one-way repeated measures Analysis of Variance (ANOVA). Mean values at a given sampling time were compared using Tukey's multiple comparison tests at $P \leq 0.05$. Statistical analysis was carried out in IBM SPSS Statistics 24.

3. Results

3.1. Available N, P, microbial biomass and redox potential in sand-straw mix

Ammonium in sand alone and sand-straw mixes on day 4 did not differ among treatments (Figure 1(a)). However, on days 8 and 16, ammonium in fresh straw was about two-fold higher than sand alone. On day 16, ammonium was two-fold higher in 7D straw than

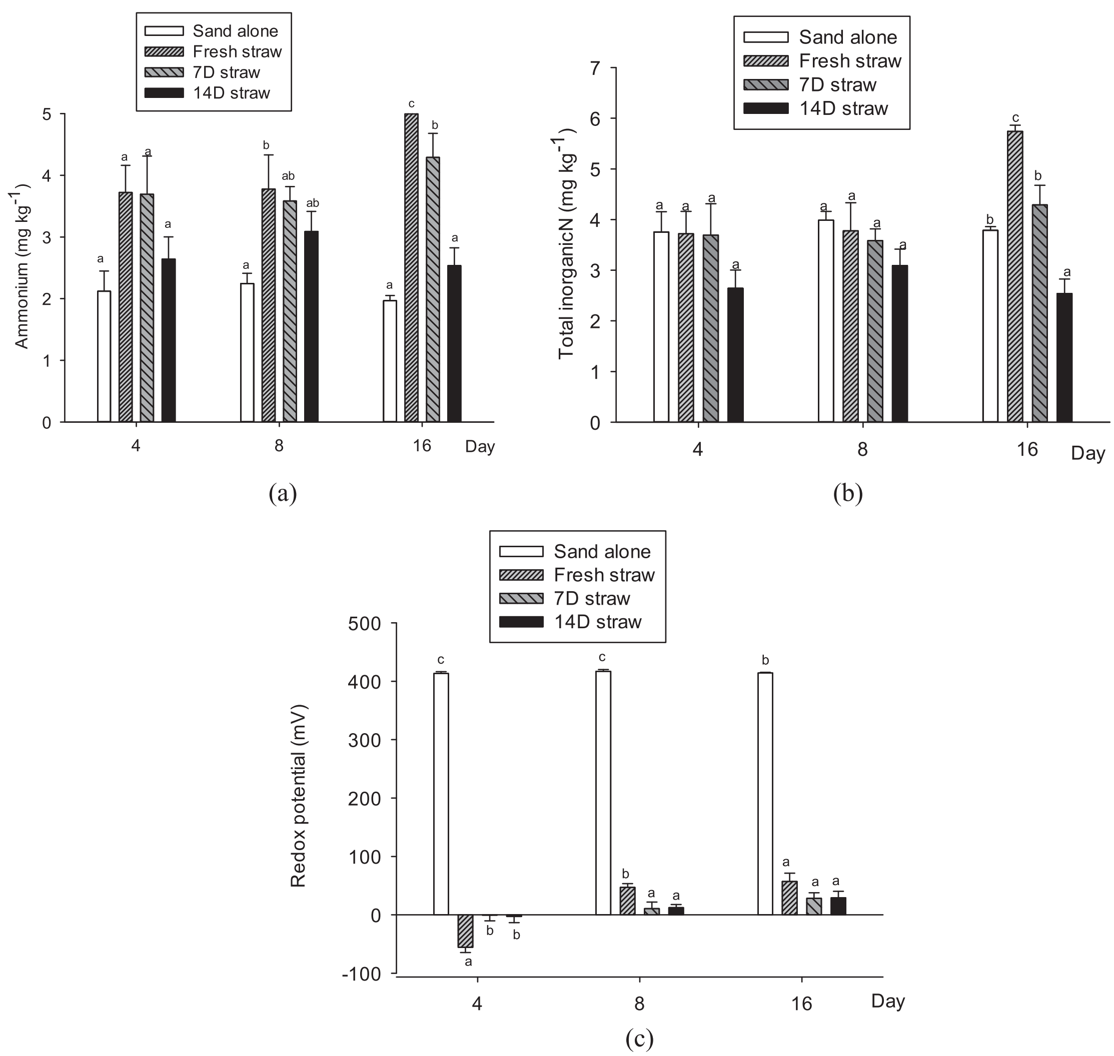

Figure 1. Ammonium (a), total inorganic N (b) after leaching on days 4, 8 and 16 and redox potential before leaching (c) in sand alone, sand mixed with fresh straw or straw decomposed for 7 or 14 prior wastewater application. At a given sampling time, columns with different letters are significantly different ($n = 4$, $P \leq 0.05$).

sand alone, but ammonium did not differ between sand alone and 14D straw throughout the experiment. Only on day 16, ammonium in fresh straw was higher than in 7D straw and 14D straw. In fresh straw and 7D straw, ammonium concentration was about 15–35% higher on day 16 than day 4. In the control and 14D straw, ammonium remained stable over time. Nitrate was not detectable in the amended treatments (Table S1). Nitrate in sand alone ranged from 1.6 to 1.8 mg kg^{-1}.

Inorganic N (sum of nitrate and ammonium) did not differ among treatments on day 4 and 8 (Figure 1(b)). On day 16, inorganic N was highest in fresh straw where it was three-fold higher than 14D straw and 30% higher than sand alone and 7D straw.

Available P in amended treatments was not detectable throughout the experiment (data not shown). In sand alone, available P ranged from 0.3 to 0.4 mg kg^{-1}. In all treatments, soil pH ranged from 6.7 to 7.3 and gradually increased over time (Figure S1a).

MBN ranged from 0.8 to 4.3 mg kg^{-1} (Table S1). On day 4 and 8, it was highest in 14D straw. However, MBN on day 16 did not differ among treatments. MBP ranged from 0.1 to 0.4 mg kg^{-1} and did not differ among treatments (Table S1).

Redox potential before leaching was around +400 mV in sand alone which was much higher than with straw (Figure 1(c)). For amended treatments, on day 4, redox potential was lowest in fresh straw where it was 20–70 times lower than in D7 and D14 straw. On day 8 and 16, redox potential in all straw treatments was slightly higher than on day 4 but still 9–40 times lower than in sand alone.

3.2. Inorganic N and P in leachate

Leachate nitrate (Figure 2(a)) in sand alone was similar to untreated wastewater and 20–350-fold higher than in amended treatments which did not differ. In amended treatments, leachate nitrate was five to ten-fold higher on day 16 than day 4, but it remained stable in sand alone.

Leachate ammonium was more than 10-fold lower than nitrate. It was similar in sand alone and 14D straw

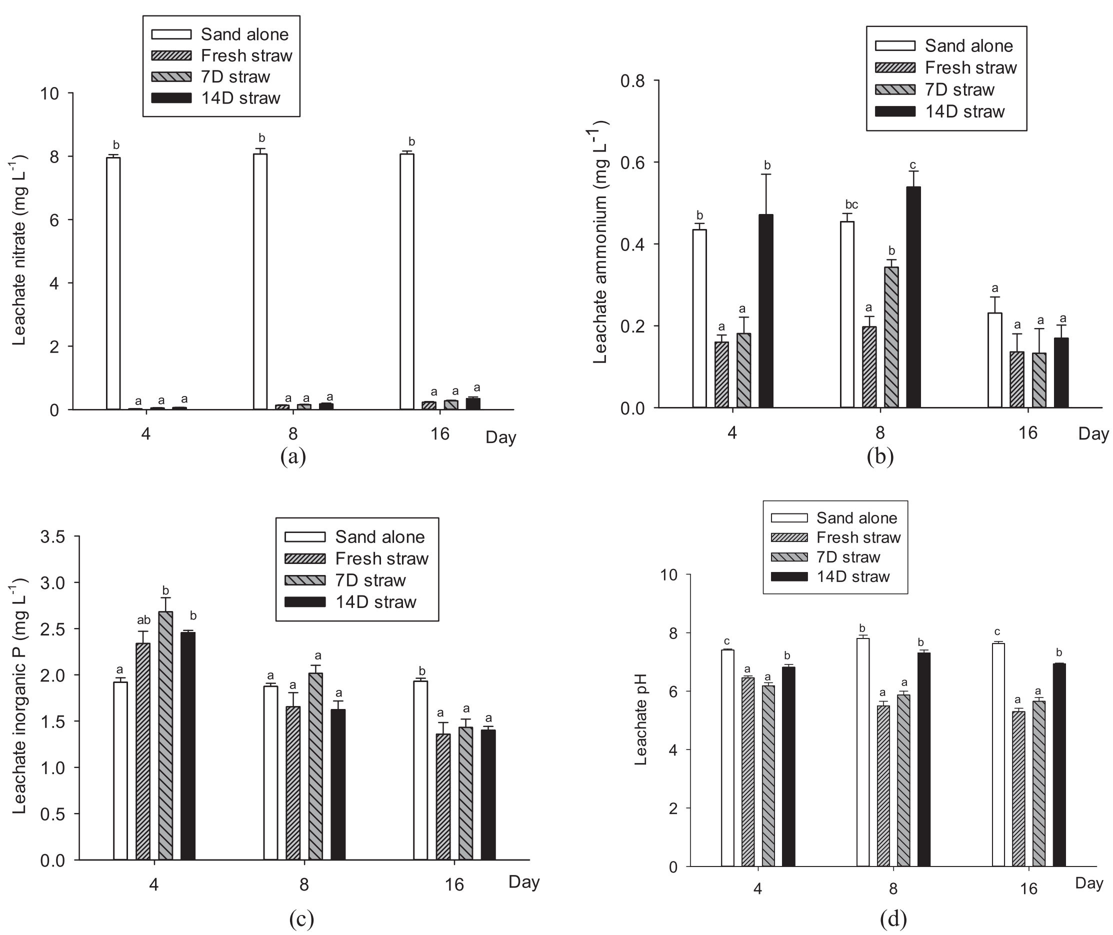

Figure 2. Leachate nitrate (a), ammonium (b), inorganic P (c) and pH (d) on days 4, 8 and 16 in sand alone, sand mixed with fresh straw or straw decomposed for 7 or 14 prior wastewater application. At a given sampling time, columns with different letters are significantly different ($n = 4$, $P \leq 0.05$).

at all leaching events (Figure 2(b)). On day 4, leachate ammonium was three-fold lower in fresh straw and 7D straw than sand alone. Leachate ammonium on day 8 was highest in 14D straw followed by 7D straw where it was about two-fold higher than in fresh straw. On day 16, leachate ammonium did not differ among treatments. Leachate ammonium was 15–64% lower on day 16 than on day 4.

Treatment differences of leachate inorganic N (Figure S1b) were similar to that of leachate nitrate. Leachate inorganic N in sand alone was 12–46-fold higher than in amended soils.

Leachate inorganic P did not differ among amended treatments (Figure 2(d)). Compared to sand alone, leachate inorganic P in 7D straw and 14D straw was about 25% higher on day 4, but did not differ on day 8. However, on day 16, leachate inorganic P was 30% lower in amended sand than sand alone. Leachate inorganic P in amended sand was 45% lower on day 16 than day 4, but remained stable over time in sand alone.

pH leachate ranged from 5.3 to 7.8 (Figure 2(e)). It was higher in sand alone than in amended soils for all leaching events. In amended treatments, leachate pH was highest in straw 14D.

3.3. Released N_2O and CO_2

Released N_2O per day was low (<0.2 ppm) and similar in amended soils for all leaching events (Figure 3(a)). Released N_2O per day was only 0.1–0.7% of total inorganic N in the column. Before leaching on day 4, released N_2O per day was about 10% higher in fresh straw and 14D straw than sand alone. It did not differ among treatments on day 8. However, before leaching on day 16, released N_2O was 15% lower in amended treatments than sand alone. For amended sand, released N_2O per day was 8–22% lower before leaching on day 16 than before day 4.

Released CO_2 per day (Figure 3(b)) in sand alone was about two to six times lower than in amended sand for all leaching events. For amended sand, released CO_2 per day was 23–67% lower in 14D straw than in fresh straw and 7D straw. Released CO_2 per day in 7D straw was about 30% lower than in fresh straw before day 4 leaching, but was similar before leaching on days 8 and 16. Compared to before day 4 leaching, released CO_2 per day before the day 16 was 13–43% higher in 7D straw and 14D straw, but 12% lower in fresh straw. It remained unchanged throughout the experiment in sand alone.

3.4. Total inorganic N and P in leaching column, the inorganic N and P in soil and leachate as percentage of inorganic N and P in the added wastewater

Total inorganic N in leaching column as percentage of inorganic N added with wastewater in sand alone was two-fold higher than in amended treatments (Table 1). For amended treatments, the percentage of total inorganic N in leaching column did not differ on days 4 and 8, but on day 16 it was lowest in 14D straw where it was about two-fold lower than fresh straw and 7D straw. The percentage of inorganic N added in the sand or sand-straw mix was similar in all treatments on

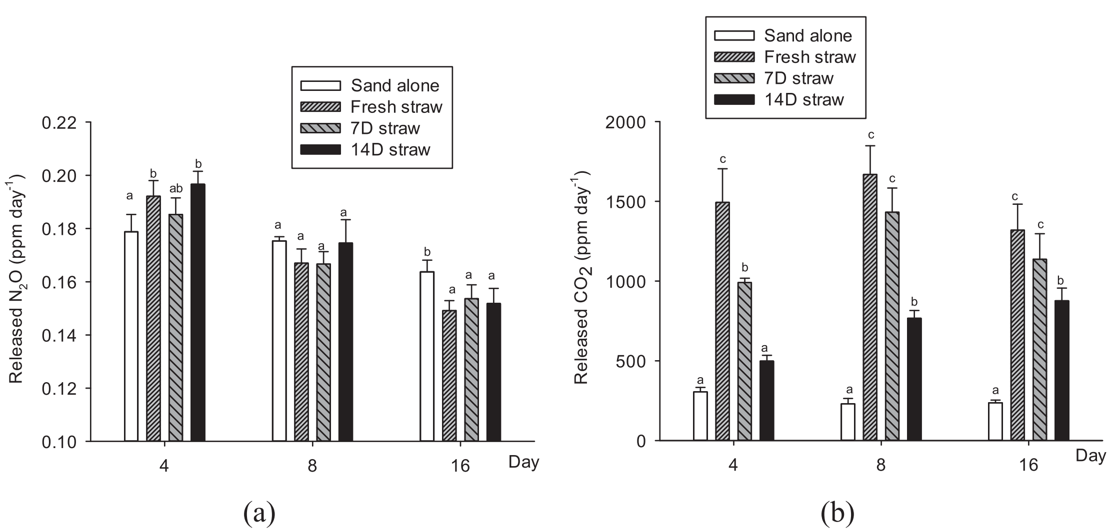

Figure 3. Released N_2O (a), CO_2 (b) in ppm day^{-1} from sand-straw mix in the two days prior to leaching on days 4, 8 and 16 in sand alone, sand mixed with fresh straw or straw decomposed for 7 or 14 prior wastewater application. At a given sampling time, columns with different letters are significantly different ($n = 4$, $P \le 0.05$).

Table 1. Total inorganic N (sum of inorganic N in straw-sand mix and leachate), inorganic N in sand and leachate on days 4, 8 and 16 as percentage of inorganic N in applied wastewater in sand alone, sand mixed with fresh straw or straw decomposed for 7 or 14 prior wastewater application. On a given day, values with different letters are significantly different ($n = 4$, $P \leq 0.05$).

	Total inorganic N			Inorganic N in sand-straw mix			inorganic N in leachate		
				Percentage of inorganic N added (%)					
Treatment	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16
Sand alone	130b	135b	127c	75a	80a	76b	55c	55b	51b
Fresh straw	76a	78a	117c	75a	76a	115c	1a	2a	2a
7D straw	75a	75a	88b	73a	72a	86b	2a	3a	2a
14D straw	57a	66a	54a	53a	62a	51a	4b	4a	3a

Table 2. Total inorganic P (sum of inorganic P in sand-straw mix and leachate), inorganic P in sand and leachate on days 4, 8 and 16 as percentage of inorganic P in applied wastewater in sand alone, sand mixed with fresh straw or straw decomposed for 7 or 14 prior wastewater application. On a given day, values with different letters are significantly different ($n = 4$, $P \leq 0.05$).

	Total inorganic *P*			Inorganic *P* in sand-straw mix			inorganic *P* in leachate		
				Percentage of inorganic *P* added (%)					
Treatment	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16	Day 4	Day 8	Day 16
Sand alone	30a	34b	28b	10a	14b	9b	20a	20a	19b
Fresh straw	29a	17a	12a	1a	0a	0a	28b	17a	12a
7D straw	30a	20a	14a	0a	0a	0a	30b	20a	14a
14D straw	33a	17a	15a	2a	0a	0a	30b	17a	15a

days 4 and 8. However on day 16, it was highest in fresh straw followed by 7D straw and sand alone and lowest in 14D straw. About 50% of inorganic N added was in leachate in sand alone. In amended treatments it was <5%.

As percentage of inorganic P added with wastewater, total inorganic P in the leaching column on day 4 did not differ among treatments (Table 2). However, it was about two-fold higher in sand alone than in amended treatments on days 8 and 16. The percentage of total inorganic P in leaching column in sand alone was stable over time, but in amended sand it was 17% lower on day 16 than day 4. Less than 2% of inorganic P added with wastewater was retained in the sand-straw mixes compared to 9–14% in sand alone. The percentage of inorganic P added as inorganic P in leachate in sand alone was about 10% lower than in amended sand on day 4, but 6% higher on day 16.

4. Discussion

Based on this study, all hypotheses; (i) N and P leaching will be lower with straw than in unamended sand, (ii) N and P removal will be higher with fresh straw amendment than with pre-decomposed straw amendments, and (iii) leachate composition and soil retention will change with incubation time, can be confirmed.

In unamended sand, leachate N concentration was higher and N removal lower than in amended soils. This can be explained by the low nutrient sorption capacity of the sand [56] and the low organic C availability to microbes in sand alone as organic C was added only with the wastewater [57,58]. This is supported by the low CO_2 release and microbial immobilization (low MBN) in sand alone. The low substrate availability also likely limited dissimilatory nitrate reduction to ammonium [59], thus most N was leached as nitrate which does not bind to soil particles because of its negative charge and is therefore easily leached [3].

In the amended soils on the other hand, nitrate concentrations in soil and leachate were much lower than sand alone suggesting that dissimilatory nitrate reduction to ammonium occurred as a result of the supply of organic C by straw [59]. This was corroborated by the higher ammonium concentration in the sand mixed with fresh straw and 7D straw compared to sand alone. N_2O release differed little between treatments and was low, indicating that denitrification was not an important process throughout the experiment. With dissimilatory nitrate reduction, N_2O release is low because nitrate is converted to ammonium [60]. CO_2 release was higher and redox potential lower in amended treatments than in sand alone. This can be explained by the substrate supply from the straw which increased microbial respiration and thus O_2 consumption.

With straw, more P was leached than sand alone on day 4. This could be due to organic acid anions produced during decomposition of wheat straw which competed with P for sorption sites on sand and straw and therefore increased P leaching [41,61]. The lower P leaching in amended sand than sand alone on day 16 on the other hand can be explained by (i) low straw decomposition rate and therefore production of organic acid anions, (ii) decomposition of organic acid anions produced earlier because they are more easily decomposable than the remaining straw, and (iii) sorption of P to the decomposed straw.

The decomposition stage of the straw influenced available N and N leaching but had little effect on N removal. Easily available organic carbon in fresh straw was decomposed initially at a high rate by soil microbes as indicated by the higher CO_2 release [62] and lower redox potential compared to pre-decomposed straw. Available ammonium was higher in fresh straw than 14D straw. This is likely because more ammonium was leached in 14D straw suggesting that functional groups produced during the later stages of decomposition have little capacity to bind ammonium. Further, on day 4, available ammonium may also be lower in 14D straw than fresh straw because of the greater N immobilization in the microbial biomass [42]. The higher CO_2 release in 7D straw than 14D straw suggests that the former was decomposed at a higher rate than 14D straw.

Although more ammonium was leached in 14D straw than fresh straw, they had similar N removal. This is likely because the main N form leached was nitrate and nitrate leaching was low in all straw amendments due to dissimilatory nitrate reduction.

5. Conclusion

This study showed that straw addition to sand resulted in two-fold greater N removal from wastewater than sand alone, irrespective of decomposition stage. This suggests that straw amendment to soil which receives wastewater inputs could strongly reduce N contamination of ground water. P removal of amended sand differed little from that of sand, indicating that straw amendment has little effect on P leaching after wastewater application. Decomposition stage of the straw had little effect on leachate inorganic N, P concentration and removal of N, P from wastewater. Release of N_2O differed little between treatments and was low indicating that denitrification was not an important process, but dissimilatory nitrate reduction was likely dominant in amended treatments throughout the experiment. Field studies are required to confirm the effect of straw addition and decomposition stage on N and P removal from wastewater. Further studies could assess the effect of different wastewater loading rates and effectiveness of the wheat straw treatment capacity over longer periods of time.

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Disclosure statement

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CHAPTER 4

EFFECT OF SHORT-TERM IRRIGATION OF WASTEWATER ON WHEAT GROWTH AND NITROGEN AND PHOSPHORUS IN SOIL

Thi Huong Xuan Le[1, 3], Luke Mosley[2], Dinh Thi Nguyen[3], Petra Marschner[1*]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia.

[2]School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

[3]Hue University of Agriculture and Forestry, Hue City, Vietnam.

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Principal Author

Name of Principal Author (Candidate)	Thi Huong Xuan Le		
Contribute to the Paper	Performed experiment, analyses of soil, wastewater and plant samples, data analysis and interpretation, wrote manuscript.		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	1/11/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

Name of Co-Author	Luke Mosley		
Contribution to the Paper	Contributed to planning of experiments, assisted in wastewater collection, discussions, proof-reading of manuscripts, supervised development of work		
Signature		Date	1/11/2019

Name of Co-Author	Dinh Thi Nguyen		
Contribution to the Paper	Supervised development of work, assisted in data analysis		
Signature		Date	01/11/2019

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, data interpretation, manuscript evaluation and correction, and acted as corresponding author		
Signature		Date	04/11/2019

Effect of short-term irrigation of wastewater on wheat growth and nitrogen and phosphorus in soil

Thi Huong Xuan Le[1, 3], Luke Mosley[2], Dinh Thi Nguyen[3], Petra Marschner[1*]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia.

[2]School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

[3]Hue University of Agriculture and Forestry, Hue City, Vietnam.

Abstract (150-250 words)

Purpose: Determine how plant age influences the effect of short-term wastewater irrigation on growth and nutrient uptake, soil available N, P concentration.

Methods: Sandy soil was left unplanted or planted with wheat and then watered with reverse osmosis (RO) water for 20 days. Wheat was planted so that plants were 7, 14 or 21 days old when half of the pots were irrigated with wastewater from day 20 to 35, the other pots received RO water until day 35. Similarly, unplanted pots received either RO or wastewater water from day 20 to 35.

Results: Irrigation with wastewater had little effect on plant dry weight, shoot N and P concentration or on available N and P, microbial biomass N and P in soil in both planted and unplanted treatments. Wastewater irrigation increased shoot N uptake compared to RO treatments only in plants that were 21 days old at the start of wastewater addition. Presence of plants reduced available nitrate up to 30-fold compared to unplanted soil.

Conclusion: In this sandy soil, short-term wastewater irrigation had little effect on wheat growth, N, P uptake and N, P concentration in soil. However, presence of plants reduced available N and P in soil compared to unplanted soils which would reduce potential of nutrient leaching after wastewater irrigation.

Keywords: inorganic N, inorganic P; growth stages; wastewater irrigation, wheat.

1. Introduction

Wastewater derived from anthropogenic activities is an environmental concern world-wide (Bedessem et al., 2005; Gibert et al., 2008). Wastewater generated from domestic, industrial and commercial activities has increased with population and economic development (Qadir et al., 2010). Reuse of wastewater for irrigation of cropland is a common practice, especially in developing countries where technologies for wastewater treatment are limited (Castro et al., 2013) and in semi-arid and arid zones where fresh water supply is scarce (Avnimelech et al., 1993; Jalali et al., 2008). According to the FAO, approximately 10% of the world's irrigated land area receives partially treated or untreated wastewater (Cooper, 2007).

Wastewater irrigation can change soil properties (Biswas et al., 2017). For example, it can reduce soil bulk density and increase soil water holding capacity, pH, EC, organic C, total N, available P and S and exchangeable cations (Na, K, Ca, Mg) compared to freshwater irrigation (Biswas et al., 2017)

However, wastewater irrigation can also result in salt and metal accumulation and nutrient leaching into ground and surface water (Avnimelech et al., 1993; Castro et al., 2013; Howarth et al., 2002; Jalali et al., 2008; Siebe & Cifuentes, 1995). Wastewater application to sandy soils is particularly problematic because sandy soils have low water holding capacity, low specific surface area for adsorption and low cation exchange capacity (Hamarashid et al., 2010). Therefore, treatment of wastewater prior to irrigation, alternative irrigation and water management practices are important to avoid imbalanced nutrient supply and mitigate the harmful effects of wastewater irrigation (Castro et al., 2013; Gardenas et al., 2005).

Wastewater can be a source of nutrients for plant growth (Siebe & Cifuentes, 1995) with the nutrient concentration depending on the source of effluent (Barreto et al., 2013; Liu & Haynes, 2011). Nitrogen is high in wastewater generated by agricultural activities (Boyer et al., 2002), while P is mostly derived from industrial and residential sources (Ruzhitskaya & Gogina, 2017). The main forms of N in wastewater are ammonium (NH_4^+-N), nitrate (NO_3^--N) and organic N (Sedlak, 1991; Sotirakou et al., 1999). Orthophosphate, polyphosphate and organic compounds are the main P forms (Sotirakou et al., 1999). Therefore, irrigation with reclaimed wastewater can increase crop yield (Meli et al., 2002) and reduce the need for chemical fertilizers which lowers production costs (Martínez et al., 2013).

Wastewater has been applied to a wide range of crops (Akhtar et al., 2012; Cereti et al., 2004). Nutrient uptake by crops can reduce the potential for nutrient leaching after wastewater irrigation (Ehdaie et al., 2010). But wastewater irrigation does not necessarily increase nutrient uptake compared to ground water irrigation (Segura et al., 2001). Nutrient uptake varies with growth stage because it depends on several factors such as nutrient demand of crops and size of root system (Ehdaie et al., 2010; Jones et al., 2011; Sattelmacher et al., 1993). Crop nutrient uptake can influence nutrient concentration in soil and leaching potential when wastewater is used for irrigation.

Little is known about the effect of short-term wastewater irrigation on early growth stages of crops and nutrient availability in soil. Short-term irrigation may be necessary in situations where there is a limited supply of wastewater. Farmers would then need to know how to maximise the effect of wastewater irrigation on plant nutrient uptake while minimizing nutrient leaching.

The aim of this study was to determine the effect of (1) wastewater irrigation at different stages of early wheat growth on wheat dry biomass, shoot N and P concentration, N and P uptake and available N and P concentration in soil, and (2) presence of wheat plants at different growth stages on available N and P concentration in soil.

The hypotheses were (1) wastewater irrigation increases wheat growth irrespective of growth stage compared to clean water irrigation, (2) N and P uptake by wheat and available N and P in soil are higher with wastewater than clean water irrigation, and (3) with wastewater irrigation, nutrient concentrations in soil are lower in planted than unplanted soil.

2. Materials and methods

2.1. Materials

As described in (Le et al., 2019), wastewater was collected from the Glenelg Sewage Treatment Plant in South Australia (Longitude 138°30'34.7"E Latitude 34°56'44.3"S). Effluent for the experiments was collected after primary sedimentation and passage through active sludge bioreactors ("SA Water wastewater treatment plants and catchments," 2013). Nitrate-N, ammonium-N and inorganic P concentrations in the wastewater (pH 7.0) were 15.8, 0.1, 2.1 mg L^{-1}, respectively.

A sandy loam from Monarto in South Australia (35º 04'S 139º 07'E) was used. The soil was air-dried and sieved to particle size < 2 mm prior to the experiment. It has the following properties: sand 74%, silt 17%, clay 9%, total P 0.38 g kg^{-1}, pH (1:5) 7.6, total organic C 6.3 g kg^{-1}, total N 1.57 g kg^{-1}, available N 14.7 mg kg^{-1}, available P 3.4 mg kg^{-1} and maximum water holding capacity (WHC) 188 g kg^{-1}.

2.2. Experimental design

There were eight treatments with five replicates each. Treatment factors were watering with reverse osmosis water (RO) or wastewater (W) from day 21, presence or absence of plants and age of plants. On day 0, 400 g soil (dry weight equivalent) was adjusted to 75% WHC before placing into 500 ml pots lined with plastic bags. This soil water content is optimal for microbial activity in soils of this texture according to a previous study using a sandy loam (Alamgir et al., 2012).

The pots were left either unplanted (UP) or were planted (P). For the planted treatments, 15 pre-germinated wheat seeds (*Triticum aestivum* L. variety Axe) were planted per pot on days 0, 7 or 14. After one week, the plants were thinned to 10 plants per pot. All pots were placed in a glasshouse with natural light where the temperature ranged from 25 to 30 ºC. From day 0 to 20, soil water content of all pots was adjusted daily by weighting and adding RO water.

From day 21 to day 35, half of the pots with wheat plants that were either 7, 14 or 21 days old were watered with wastewater (W).

The treatments are referred to as P7-W, P14-W, P21-W. The other half was watered with RO water, referred to as P7-RO, P14-RO, P21-RO. The unplanted pots were used to assess the effect of added wastewater on soil nutrient concentration over time in absence of plants. The unplanted pots were watered with RO water (UP-RO) from day 0 to 35 or received RO water until day 20 and then wastewater from day 21 to day 35 (UP-W). The same amount of wastewater was added daily to the respective pots (7.7 ml day^{-1}) with a total application of 115 ml.

On day 36, the plants were harvested, roots were carefully removed from the soil and washed. Then, soil in all treatments was destructively sampled to determine available N (ammonium, nitrate), available P, pH, microbial biomass N (MBN) and microbial biomass P (MBP).

2.3. Analyses

Analyses of soil texture, pH, maximum water holding capacity, total organic carbon, nitrogen, phosphorus, available N (ammonium, nitrate), available P extraction and microbial biomass N and P were carried out as described in Marschner et al. (2015) (Table 1).

Shoot and root dry weight were determined after drying at 55 ºC for 48 h. Inorganic N (nitrate and ammonium) and P in the applied wastewater were determined using the same colorimetric methods as for available N and available P.

2.4. Statistical analysis

After confirming normal distribution, the data of planted pots including shoot and root dry weight, available N and P, MBN and MBP in soil and leachate inorganic N and P was analysed by two-way Analysis of Variance (ANOVA) with age of plants and water source as factors. In unplanted soil, differences between water sources were tested by t-test. For a given water source, data of unplanted soil and planted treatments were compared by t-test. Statistical analysis was carried out in IBM SPSS Statistics 24.

3. Results

Shoot dry weight increased with the age of plants at onset of wastewater addition. Compared to P7-W (7 days of growth prior to wastewater addition), shoot dry weight was two-fold higher in P14-W (14 days prior growth) and five-fold higher in P21-W (21 days prior growth) (Figure 1a). Root dry weight was lowest in P7 where it was about two-fold lower than in P14 and P21 (Figure 1b). There was no significant difference in shoot and root dry weight between W and RO water treatments.

Shoot N and P concentration generally decreased with age of plants. Compared to P7-W, shoot N concentration was 23% lower in P14-W and 50% lower in P21-W (Figure 1c). Shoot P concentration was highest in P7 treatments where it was 30-40% higher than in P14 and P21 treatments (Figure 1e). Shoot N and P concentration did not differ between W and RO treatments.

Shoot N and P uptake generally was lowest in P7 treatments. Shoot N uptake in P7 was 30-40% lower than in P14 and P21 (Figure 1d). Shoot N uptake differed between W and RO only in treatments P21 where it was 23% higher in P21-W than P21-RO. Shoot P uptake was about 10-fold lower than N uptake (Figure 1f). It was highest in P21 where it was about 40% higher than in P7 and 20% higher than in P14. Shoot P uptake differed between W and RO treatments only in P7 where P7-W was 18% higher than in P7-RO.

Available nitrate in planted soils was low (≤ 2.1 mg kg^{-1}) and did not differ between RO and W treatments (Table 2). In unplanted soil, available nitrate was 10% higher with W than with RO water. Nitrate in unplanted soil was 15 - 30 times higher than in planted soil. Available ammonium was not affected by plant age at the onset of wastewater irrigation (Table 2). Available ammonium in both planted soil and unplanted soil was low (≤ 1.4 mg kg^{-1}) and did not differ between treatments with RO and with W. Available ammonium in planted soil was 20% higher than in unplanted soil in the RO treatments, but was not affected by plants in the W treatments. Available P was 24% higher in P7-W than P14-W (Table 2). It did not differ between W and RO treatments. Available P in unplanted soil was about 25% higher than in planted soil.

MBP and MBN were not affected by the age of the plants prior to wastewater irrigation. MBP did not differ between W and RO treatments (Table 2). MBN was not affected by the source of the irrigation water except in P21 where it was 20% higher in P21-RO than in P21-W (Table 2). MBN and MBP in planted soil were about 20% higher than in unplanted soil,

Soil pH (Table 2) ranged from 7.7 - 8.4 and was not affected by plant age. It differed little between W and RO treatments. Soil pH was about 0.3 – 0.6 units higher in planted than unplanted soil.

4. Discussion

Based on this study, some hypotheses can be confirmed but not for all measured parameters. In this experiment, 4.5 mg kg^{-1} inorganic N (with > 95% as NO_3^--N) and 0.6 mg kg^{-1} inorganic P were added with wastewater (115 ml). This wastewater addition was not enough to increase plant growth compared to RO irrigation in this sandy soil which was relatively in high available N and P. Therefore, the first hypothesis (wastewater irrigation increases growth of wheat plants irrespective of growth stage compared to clean water irrigation) is declined.

Wastewater irrigation increased N uptake by about 20% in P21-W compared to P21-RO, but had no effect on N uptake in the plants that were younger when irrigated with wastewater. This is likely due to a greater root system of three-week old plants which allowed them to access more nutrients from soil that received wastewater than the younger plants. Previous studies have also shown that root biomass and plant growth rate influence N uptake (Ehdaie et al., 2010; Gastal & Lemaire, 2002).

However, there was little difference in soil available N and P between the wastewater and RO treatments. This suggests that mineralisation of N and P of native soil organic matter was much greater than inorganic N and P added with wastewater. Hence, the second hypothesis (N and P uptake by wheat and available N and P in soil are higher with wastewater than clean water irrigation) can only be confirmed for N uptake of the three-week old plants.

On the other hand, soil available N and P were lower in planted than unplanted soil. This is caused by nutrient uptake of both wheat plants and soil microorganisms in planted soil. Previous studies also showed that plants reduce nutrient leaching (Ehdaie et al., 2010; Gastal & Lemaire, 2002). In this study we showed that this already occurs in young plants. Wheat plants absorb nutrients from soil for growth, reducing the nutrient concentration in soil. Further, plants also provide organic C (as roots and exudates) for microbes leading to higher microbial biomass N and P in planted than unplanted soil. The lack of difference in MBN and MBP between RO and W treatments suggests that microbes took up mainly N and P mineralised from the native SOM. Hence, the third hypothesis (with wastewater irrigation, nutrient concentrations in soil are lower in planted than unplanted soil) can be confirmed for both wastewater and RO water irrigation. This suggests that the presence of even young plants can significantly reduce the risk of nutrient leaching after wastewater irrigation.

In previous studies we showed that addition of wheat straw to sand leached with wastewater can reduce nitrate leaching (Le & Marschner, 2018; Le et al., 2019). In sand with wheat straw leachate nitrate was at least 60-fold lower than in unamended soil. The reduction can be explained by dissimilatory nitrate reduction to ammonium and ammonium sorption to wheat straw. Hence the results of this and our previous studies suggest that inclusion of suitable crops and/or organic amendments should be considered when wastewater is applied to sandy soils. Field trials could be undertaken to confirm these effects, including assessing nutrient retention over a longer time period than in this laboratory study.

The effect of wastewater irrigation on plant growth, available N and P may vary with soil type. It may increase plant growth in a nutrient-poorer soils which have insufficient nutrient available to plants. Further, the impact of wastewater on soil nutrient concentrations and leaching potential depends on a number of factors, such as quality of the wastewater, soil characteristics and type of irrigated crops (Mojid & Wyseure, 2013) as well as length of application.

5. Conclusion

This study showed that short term wastewater irrigation had little effect on wheat plant biomass, soil MBN, MBP, available N and P, but increased N uptake of older plants compared to RO water irrigation. It increased shoot N uptake only in plants that were 21 days when wastewater irrigation started, likely because only the older plants had sufficient roots to take up N from wastewater. Nutrient uptake by older plants and soil microorganism strongly reduced N availability in soil and would therefore reduce the risk of N leaching.

Further studies are required to investigate the effect of wastewater irrigation on wheat plant growth and leaching potential in nutrient poorer soils. In addition, studies on metal accumulation in soil and wheat plants after wastewater irrigation are needed to evaluate benefits and risks of wastewater irrigation.

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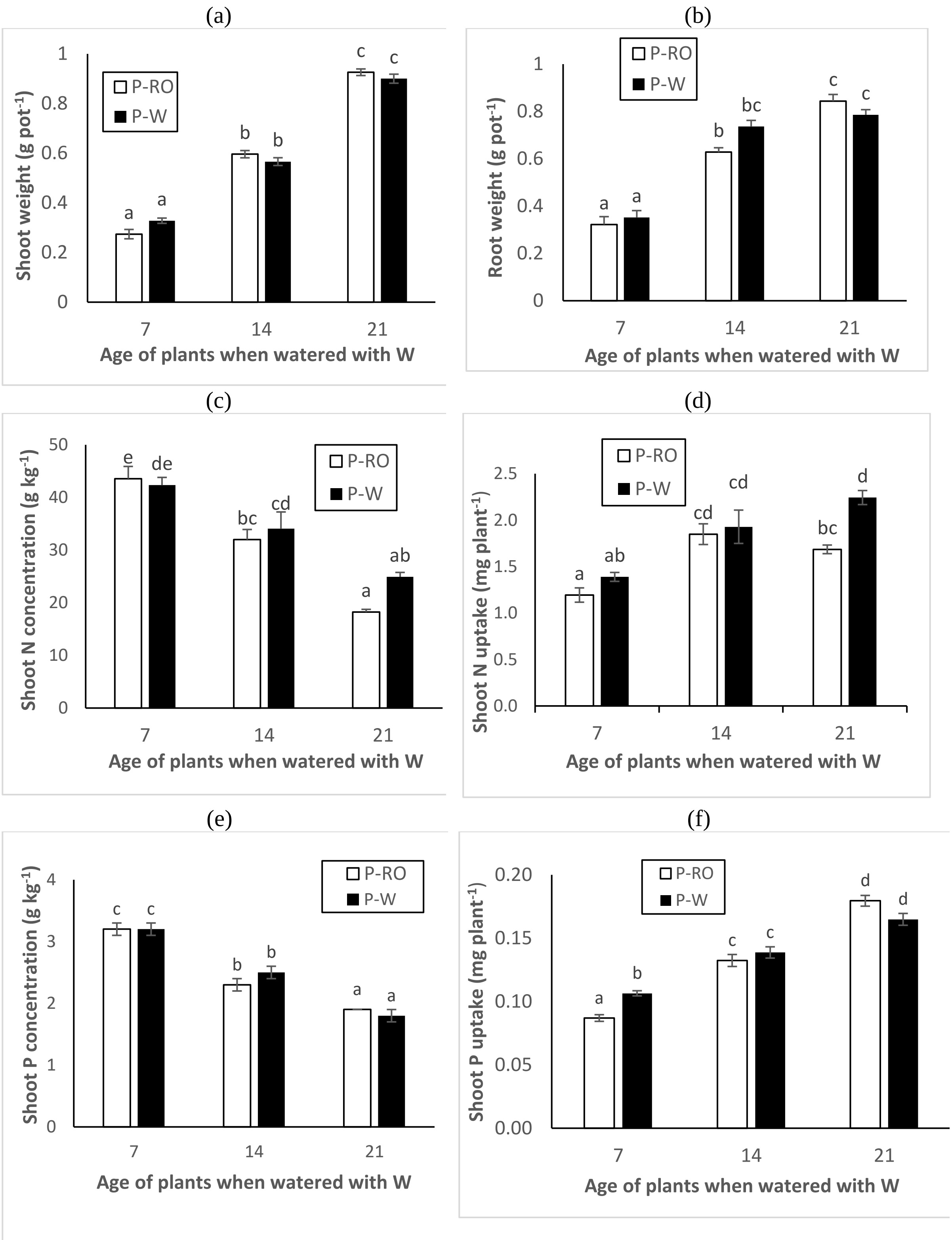

Figure 1. Shoot dry weight (a), root dry weight (b), shoot N concentration (c), shoot N uptake (d), shoot P concentration (e) and shoot P uptake (f) of 7, 14 and 21 day-old wheat watered with reverse osmosis water (P-RO) or with wastewater (P-W) for 14 days. Bars with different letters are significant differences between treatments (age of plants x water source) ($n = 5$, $P \leq 0.05$).

Table 1. Analyses as described in Marschner et al. (2015)

Parameter	Details	Reference
Soil texture	Hydrometer method	Gee and Or (2002)
Soil pH	1:5 soil:water ratio, 1 h shaking	Rayment and Higginson (1992)
Soil maximum water holding capacity	At matric potential -10 kPa	Wilke (2005)
Total organic C	Wet oxidation and titration	Walkley and Black (1934)
Total N	Digestion with H_2SO_4, measurement by modified Kjeldahl method	Bremner and Mulvaney (1982)
Total P	Digestion with 1:3 HNO_3 and HCl, measurement by phosphovanado-molybdate	Hanson (1950)
Available N extraction	2 M KCl at a 1:10 soil extractant ratio, 1 h shaking	
Ammonium N		Willis et al. (1996)
Nitrate N		Miranda et al. (2001)
Available P extraction	Anion exchange resin	Kouno et al. (1995)
Available P measurement		Murphy and Riley (1962)
Microbial biomass extraction	Chloroform fumigation and extraction with 0.5 M K_2SO_4	Vance et al. (1987)
Microbial biomass N	Ammonium N in extract, biomass N= (fumigated-unfumigated) x 0.57	Moore et al. (2000)
Microbial biomass P	Anion exchange resin with hexanol, biomass P = fumigated-unfumigated	Kouno et al. (1995)

Table 2. Nitrate, ammonium, total inorganic N, available P, microbial biomass N (MBN) and P (MBP) and soil pH before leaching in soil watered with reverse osmosis water or with wastewater that was unplanted (UP-RO and UP-W) and or planted with 7, 14 and 21 day old wheat (P-RO and P-W) (n = 5, P ≤ 0.05). In planted soil, different letters indicate significant differences treatments (age of plants x water source). In unplanted soil different letters indicate significant differences between reverse osmosis (RO) and wastewater (W) treatments. For a given water source, asterisk (*) indicates significantly higher value and hash (#) indicates significantly lower value in planted compared to unplanted treatments

Age of plants when watered with W	Planted/ unplanted	Nitrate ($mg\ kg^{-1}$)		Ammonium ($mg\ kg^{-1}$)		Available P ($mg\ kg^{-1}$)		MBP ($mg\ kg^{-1}$)		MBN ($mg\ kg^{-1}$)		Soil pH	
		RO	W	RO	W	RO	W	RO	W	RO	W	RO	W
7	P7	2.1b#	1.5ab#	1.2a	1.2a	3.2ab#	3.7b	3.7a*	3.2a	5.6a*	5.2a*	8.3ab*	8.3ab*
14	P14	1.1a#	1.3ab#	1.3a*	1.2a	3.0ab#	2.8a#	3.8a*	3.7a*	5.7ab*	5.4a*	8.2ab*	8.2ab*
21	P21	1.1a#	1.1a#	1.4a*	1.3a	2.8a#	3.1ab#	3.8a*	3.6a*	6.6b*	5.3a*	8.2a*	8.4b*
	UP	29.4a	32.2b	1.1a	1.1a	4.2a	3.9a	2.8a	3.1a	4.5a	4.4a	7.7a	7.9b

CHAPTER 5

CONCLUSION AND FUTURE RESEARCH

Discharge of untreated wastewater to receiving water bodies is an environmental problem because it can contain high concentrations of nutrients and pollutants (Bedessem et al., 2005). Therefore, treatments are needed that minimise nutrient leaching from soils receiving untreated wastewater. Organic amendments can with low nutrient concentrations such as cereal straw are used in wastewater treatment plants to stimulate microbial nutrient uptake or electron donor for denitrification (Ashok & Hait, 2015) and dissimilatory nitrate reduction to ammonium (Burgin & Hamilton, 2007). However, little is known about the effect of wheat straw amendment to sandy soil at different addition rates and decomposition stages on inorganic N and P removal from wastewater. In this thesis the effect of different amendment rates and decomposition stages of wheat straw on removal of inorganic N and P from wastewater was studied in leaching column experiments. Wastewater may also be a valuable source of nutrients for plants (Siebe & Cifuentes, 1995) but can cause nutrient leaching (Castro et al., 2013), particular in sandy soils which have low water and nutrient retention capacity (Farrington & Campbell, 1970). Little is known about the effect of plant age on soil nutrient availability after wastewater application. The effect of different growth stages of wheat plants on soil available N and P when wastewater was used for irrigation was assessed in a pot experiment.

In the first leaching column experiment (Chapter 2) (Le et al., 2019a), the effect of addition rates of wheat straw on inorganic N and P in leachate and nutrient availability in sandy soil following exposure to wastewater for different lengths of time was assessed. Sand alone or amended with wheat straw at 2.5, 5, 7.5, 10, 12.5 g wheat straw kg^{-1} was covered with wastewater. Leaching was carried out after 4, 8 and 16 days. Compared to the unamended control, nitrate in soil and leachate was lower in amended soils, whereas ammonium in soil was higher. N_2O oxide emission was low which suggested that dissimilatory nitrate reduction to ammonium was occurring in amended treatments due to the supply of organic C by straw (Liu et al., 2016), followed by binding of ammonium to the soil and/or residual straw. Straw addition had little effect on P retention and leaching. Straw addition increased CO_2 release and reduced the redox potential compared to unamended sand, suggesting the substrate supply from the straw increased microbial respiration and consumption of O_2 and other electron acceptors (e.g. nitrate). In conclusion, wheat straw can remove large proportions of inorganic N from wastewater irrespective of straw rate.

Straw rates had little effect on removal of inorganic N and P from wastewater. However, decomposition of wheat straw soil amendments before application of wastewater may influence its capacity to remove N and P. This is because decomposition of wheat straw leads to changes in chemical properties and decomposability.

The study described in Chapter 3 (Le et al., 2019b) aimed to investigate the influence of decomposition stages of the straw and the duration of contact with wastewater on inorganic N and P in the sand-straw mix and leachate. Wastewater was added either to fresh straw or straw that had been incubated moist in sand for 7 or 14 days (7D or 14D straw). Leaching was carried out 4, 8 or 16 days after addition of wastewater. Wheat straw addition reduced leaching of inorganic N and P compared to sand alone, but the decomposition stages of the straw had little effect on removal of inorganic N and P from wastewater and had no consistent effect on microbial biomass N and P. Inorganic N in the sand-straw mix was higher in fresh straw than in 14D straw on day 16. This suggests that functional groups produced during later stages of decomposition (14D straw) lowered capacity to bind ammonium. CO_2 release was higher with fresh than in pre-decomnpsed straw. It can be concluded that wheat straw amendment to sand can reduce N and P leaching irrespective of decomposition stage.

In both leaching column experiments, very little N_2O was released suggesting that denitrification was not an important process, consistent with the findings of the first column leaching experiment.

In the pot experiment described in Chapter 4 (Le et al., 2019c) sandy soil was left unplanted or planted with wheat and then watered with reverse osmosis (RO) water for 20 days. Wheat was planted so that plants were 7, 14 or 21 days old when half of the pots were irrigated with wastewater from day 20 to 35, the other pots received RO water until day 35. Similarly, unplanted pots received either RO or wastewater water from day 20 to 35. On day 35, soil was leached with RO water to assess potential nutrient leaching. The results showed that irrigation with wastewater had little effect on wheat plant biomass, shoot N and P concentration, soil MBN, MBP, available N and P concentrations, but increased shoot N uptake in older plants compared to irrigation with RO water. This is likely because only the older plants had sufficient roots to take up N from wastewater. Further, nutrient uptake by older plants and soil microorganism may reduce the risk of N and P leaching.

It can be concluded that amendment of sandy soil with wheat straw can improve N retention and reduce N leaching. Similarly, nutrient leaching can be reduced by growing plants, particularly when the plants have well-developed root systems. A large root system can also improve plant N uptake from wastewater.

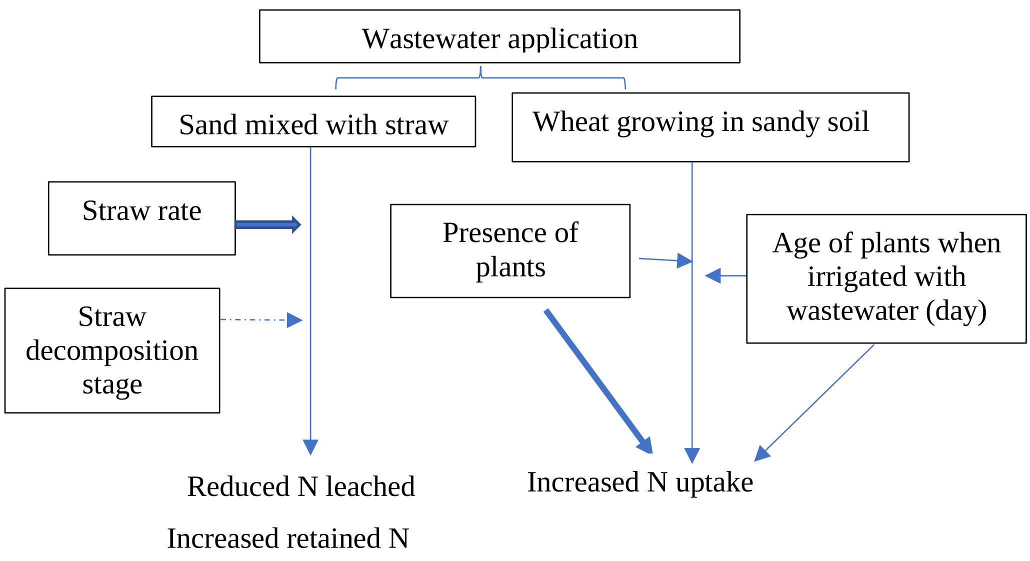

Figure 3. Conceptual diagram of the results of this thesis which examined the fate of inorganic N and P of wastewater added to sand mixed with wheat straw and wheat growing in sandy soil.

This research provided information on using wheat straw amendment to sand or growing wheat plant on sandy soil to remove inorganic N and P from wastewater. However, this research has also generated a number of questions which could be addressed in the future to maximize the use of wheat straw or wheat plants to reduce leaching after wastewater irrigation.

The studies in this project were conducted on a small scale and relatively short timeframe in leaching columns and pots in a glasshouse. To ensure that the results are applicable in the field, the following field studies are recommended to be carried out:

- Field trial with wheat straw amendment and wheat plants with different wastewater irrigation rates. Irrigation rate will affect residence time of the wastewater in the soil, leaching rate and likely the leachate composition.
- Trial various wheat plant sowing densities at the field trial site as this will influence plant nutrient uptake and also leaching rate.
- Trial using wastewater from different stages in the wastewater treatment plant process as this will affect the composition of the wastewater applied to the soil and potentially the treatment efficiency.
- Longer term repeated application of wastewater to determine treatment capacity and the point at which wheat straw needs to be replenished or wheat plants harvested.

In such field experiments, soil and plant samples could be collected at different times. Plant samples could be analysed for nutrient and heavy metals, soils for available nutrients and metals. Leachate could be collected with suction cups or multi-level piezometers inserted at different soil depths and analysed for nutrients and other contaminants. If such experiments were continued for several years, the long-term effect of wastewater addition could be assessed.

In this thesis, wheat straw was decomposed before wastewater addition, but only for up to 16 days. In the field, straw may decompose for longer periods of time (several weeks or months) before the next crop is sown and irrigated with wastewater. During such a longer period of decomposition, abundance of functional groups and straw amount may change which could affect nutrient retention. Therefore, experiments with longer decomposition periods before wastewater addition should be carried out.

Nutrients retained by the straw may be mobilised when wastewater irrigation is followed by rain. Nutrient retention and loss could be investigated either in lab experiments where rain is simulated by addition of RO water or in the field following rain.

In the pot experiment described in Chapter 4, wastewater addition had no effect on plant growth and little effect on plant nutrient uptake. However, wastewater may increase plant growth and nutrient uptake when added to a nutrient-poor soil. Experiments could be carried out with soils differing in nutrient availability to determine the threshold soil nutrient availability below which wastewater increases plant nutrient uptake.

Sandy soils are sometimes ameliorated by addition of clay soils because clay has a high retention capacity for cations and also phosphate. Addition of clay soil to sandy soils has been shown to increase organic matter content and nutrient retention. The effect of nutrient retention and leaching with wastewater irrigation could also be assessed. Unlike organic amendments, clay addition could provide a long-term improvement of nutrient retention in sandy soils.

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Appendix

MIXING ORGANIC AMENDMENTS WITH HIGH AND LOW C/N RATIO INFLUENCES NUTRIENT AVAILABILITY AND LEACHING IN SANDY SOIL

Thi Huong Xuan Le[1, 2], **Petra Marschner**[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia

[2]Hue University of Agriculture and Forestry, Hue City, Vietnam.

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Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Thi Huong Xuan Le		
Contribute to the Paper	Performed experiment, analyses of soil, wastewater and plant samples, data analysis and interpretation, wrote manuscript.		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	21/08/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, data interpretation, manuscript evaluation and correction and acted as corresponding author.		
Signature		Date	21/08/2019

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RESEARCH ARTICLE

Mixing organic amendments with high and low C/N ratio influences nutrient availability and leaching in sandy soil

Thi Huong Xuan Le[1, 2] and Petra Marschner[1*]

[1]*School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia*
[2]*Hue University of Agriculture and Forestry, Hue City, Vietnam.*
**Corresponding author:* petra.marschner@adelaide.edu.au

Abstract:

Little is known about available N and P, microbial biomass and leachate N and P concentration in mixes of organic materials differing in C/N ratio. Sandy soil was amended with wheat straw (W, C/N 71) and cow manure (CM, C/N 7) either alone (100W and 100CM) or in different ratios (values are weight percentage of the organic materials): 75W-25CM, 50W-50CM, 25W-75CM. The control was unamended soil. Moist soil was incubated for 26 days, leaching was carried out on days 10 and 25. Soil was sampled on day 9 (before first leaching) and day 26 (after the second leaching). Cumulative respiration over 26 days was similar in the unamended control and 100CM, it increased with proportion of W in the amendments. Per kg of soil, amended soils did not differ in available N, microbial biomass N (MBN) and leachate inorganic N concentration, but available P and leachate inorganic P increased with proportion of CM in the amendment. However, available N, MBN and inorganic N in leachate per g N added was highest in 100W and lowest in 100CM. In contrast, available P and leachate P concentration per g P added increased with proportion of CM. Measured available N and leachate inorganic N were lower than expected values whereas measured available P and inorganic P in the leachate were higher than expected. In mixes, CM appears to reduce N mineralisation in wheat whereas W stimulates P release from cow manure.

Keywords: Available N; available P, leached N, leached P; cow manure, organic amendment mixtures, wheat straw.

1. Introduction

Organic amendments can be used as nutrient source for plants, but their effect on nutrient availability depends on properties such as C/N ratio and concentration of rapidly and slowly decomposable compounds (Palm and Sanchez, 1991; Berg and McClaugherty, 2004; Partey *et al.*, 2013; Zheng and Marschner, 2017). Mixing of organic amendments differing in chemical composition allows manipulating decomposition, mineralization and leaching. Incorporation of a high C/N ratio together with a low C/N ratio organic material can lead to lower rates of N mineralization, thereby reducing N leaching compared to low C/N ratio material alone (Vityakon *et al.*, 2000). Partey *et al.* (2013, 2014) showed that mixing maize (*Zea mays*) residues with high C/N ratio (37) with either *Tithonia diversifolia* (C/N ratio 14) or *Vicia faba* (C/N ratio 8) residues at a 1:1 ratio (w/w), tripled decomposition and N release rate compared to that of maize alone. Similarly, Singh *et al.* (2007) found that mixing Sesbania (C/N 16) with wheat straw (C/N 82) doubled decomposition rate and increased N availability and microbial biomass compared to wheat straw alone.

However, decomposition and nutrient release in mixes are not always predictable based on that of the individual materials (Handayanto *et al.*, 1997). Predicted values are calculated from mineralisation of individual species multiplied by their proportion in the mix. Interactions among species in mixes can lead to additive and non-additive responses, with non-additive responses being observed more often (Gartner and Cardon, 2004). In an additive response, measured mineralisation in the mix matches predicted values, whereas in a non-additive response, measured mineralisation in mixes is either lower or higher than expected values (Gartner and Cardon, 2004). Interactions between organic materials in mixes may depend on parameter studied. In mixes of four litter types, Bonanomi *et al.* (2010) showed that measured values of mass loss, N, Ca contents were lower than expected whereas measured values of K and Mg contents were higher. There are several factors driving the interaction among different organic materials in mixes, among which chemical composition is considered to be particularly important (Chapman *et al.*, 1988). For example, nutrients released from decomposable material can accelerate decomposition rate of less decomposable material in mixes in a synergistic response (Chapman *et al.*, 1988). On the other hand, inhibiting compounds such as phenolics and tannins in one material can retard decomposition of other materials (Dix, 1979). Further, chemical, physical and biological changes in mixes can influence the outcome of interactions in mixes (Gartner and Cardon, 2004).

Previous studies have focused on the effect of mixing of different organic materials on mass loss and nutrient contents (e.g., Xiang and Bauhus, 2007; Bonanomi *et al.*, 2010), but little is known about the effect of mixing of organic materials on microbial biomass, cumulative respiration and leachate nutrient concentration compared to expected values. The aim of this study was to determine the effect of organic amendment with different C:nutrient ratio (wheat straw with high C:nutrient ratio and cow manure low C:nutrient ratio) added singly or as mix on available N, P, microbial biomass N (MBN) and microbial biomass P (MBP) in a sandy soil and on inorganic N and P in leachate. Sandy soil was chosen to minimize sorption of nutrients to soil and thereby maximizing availability and leaching. The following hypotheses were tested: (i) due its low N and P concentration compared to cow manure, addition of wheat straw will result in lower N and P availability and N and P in leachate than with cow manure, (ii) in mixes, non-additive interactions will dominate, but depend on parameter assessed.

2. Materials and Methods

2.1. Sandy soil

A coarse sand from a sand pit in South Australia was used (Santos Ready Mixed Concrete Pty Ltd). The sand was air-dried at room temperature and sieved to particle size < 2 mm. The sand was incubated at 80% of water holding capacity (WHC) for 10 days at room temperature to activate soil microbes before mixing with organic materials. Butterly *et al.* (2010) showed that 10 days after rewetting air-dry soil, soil respiration is stable. For soil analyses, see section 2.4.

2.2. Organic materials

Two types of organic materials were used: mature wheat straw (*Triticum aestivum* L.) and cow manure (Table 1), referred to as W and CM. The organic materials were oven-dried at 40 °C, ground and sieved to particle size from 0.25 to 2 mm. For analyses of properties, see section 2.4.

2.3. Experimental design

The amendments were used alone (100W and 100CM) or mixed at different ratios (percentage in mix): 75W-25CM, 50W-50CM, 25W-75CM. Then, the organic materials were thoroughly mixed into the sandy soil at the rate 10 g dry weight kg^{-1} dry soil. Total N, P and C in each treatment were calculated based on total N, P and C of W and CM and their proportion in each treatment. The control was unamended sand. The sand-organic material mixture (30 g) was placed in PVC cores (1.85 cm radius, 5 cm height) and incubated at 80% WHC in the dark at 22-25 °C. A preliminary experiment had shown that soil respiration was maximal at this water content in this soil. Leaching was carried out on days 10 and 25 with four replicates per treatment and leaching event. Cores were destructively sampled on day 9 (before the first leaching) and day 26 (after the second leaching) for determination of available N, P and MBN, MBP. The cores to be sampled on day 9 were placed in 1L glass jars for respiration measurement until day 9. The other set of cores was incubated on trays in the same environmental conditions as the cores in the jars. The cores on the trays were leached on day 10, dried as described below and placed into the glass jars for respiration measurement until the second leaching on day 25. The following day (day 26), the soil in the cores was destructively sampled. Leachate was analyzed for inorganic N, inorganic P and water extractable organic carbon. The water content was maintained at 80% WHC throughout incubation by adding reverse osmosis (RO) water by weight.

For leaching, 50 ml RO water was added in five 10 ml aliquots. Between additions, the water was allowed to drain from the soil surface before the next aliquot was added. This amount of RO water was used to obtain sufficient leachate for the analyses (42–45 ml). After the first leaching, the cores were placed in an oven at 40 °C for about 4 h until the water content was 80% WHC. The expected value for the measured parameters in the organic material mixes was calculated based on average concentration of separate organic amendment according to the following equation (Gartner and Cardon, 2004):

Expected value = (Proportion of W in mix * concentration in sole W) + (Proportion of CM in mix*concentration in sole CM). Equation 1

2.4. Analyses

Soil texture was determined by the hydrometer method (Gee and Or, 2002). Soil pH and electrical conductivity were determined in a 1:5 soil: water extract after 1 hour end-over-end shaking at 25 °C (Rayment and Higginson, 1992). WHC of the sand alone or amended with the organic materials was measured using a sintered glass funnel connected to 100 cm water column (matric potential -10 kpa) as described by Wilke (Wilke, 2005). With organic materials, WHC was about three-fold higher than sandy soil alone. Total organic carbon in soil and residues was determined using the dichromate oxidation and titration method (Walkley and Black, 1934). For measurement of total N, residues were digested in H_2SO_4, then total N in the digest was measured by a modified Kjeldahl method (Vanlauwe *et al.*, 1996). For total P, residues were digested in HNO_3 and $HClO_4$ (6:1). Total P in the digest was measured by the phosphovanado-molybdate method (Hanson, 1950).

Due to the low respiration rate of the sand-organic material mixes and the detection limit of the gas analyser (2% CO_2), the CO_2 concentration in the headspace of the jars was measured every two days using a Servomex 1450 infrared gas analyser (Servomex, UK) as described in Setia *et al.* (2011). After each CO_2 measurement (t1), the jar was opened to release the air from the headspace using a fan followed by determination of the CO_2 concentration (t0). The CO_2 respired every interval was calculated as the difference in CO_2 concentration between t1 and t0.

Available N was measured after 1 h end-over-end shaking with 2 M KCl at a 1:5 soil extractant ratio. The nitrate-N concentration in the extract was measured after Miranda *et al.* (2001), the ammonium concentration after Willis *et al.* (1996). Available N was measured colorimetrically at 450 and 685 nm for nitrate and ammonium.

Available P was extracted by the anion exchange resin method (Kouno *et al.*, 1995), and P concentration was determined colorimetrically at 712 nm (Murphy and Riley, 1962).

MBN was extracted by the fumigation extraction method (Vance *et al.*, 1987). Soil samples were fumigated with chloroform for 24h followed by shaking with 0.5 mol L^{-1} K_2SO_4 at 1:4 soil extractant ratio for 1h. For MBN determination, ammonium-N was determined in the extract (Moore *et al.*, 2000). MBN was calculated as the difference in ammonium-N concentration between fumigated and non-fumigated samples divided by 0.57 as suggested by Moore *et al.* (2000).

MBP was determined by the anion exchange method of Kouno *et al.* (1995) with the modification that hexanol was used for fumigation instead of chloroform. P concentration was determined colorimetrically as described above for available P (Murphy and Riley, 1962). MBP was calculated by subtracting the P content of fumigated from un-fumigated samples.

Inorganic N, P in the leachate were determined using the same analytical methods as for available N, available P. Organic C in the leachate was determined by oxidising with $K_2Cr_2O_7$ and H_2SO_4. The remaining $K_2Cr_2O_7$ was titrated with acidified $(NH_4)_2Fe(SO_4)_2.6H_2O$ (Anderson and Ingram, 1993).

Cumulative respiration, available N, P, MBP and MBN, leached inorganic N and P and organic C were expressed per g or kg of soil or per g C, N and P added. Expression per g nutrient added can be used to assess its availability/decomposability which is particularly useful in mixes of organic materials.

2.5. Statistical analysis

The data of available N, P, MBN and MBP in soil and inorganic N, P and WEOC in leachate was analysed by one-way repeated measures Analysis of Variance (ANOVA). Cumulative respiration was analysed by one-way ANOVA. Mean values of four replicates at a given sampling time were compared using Tukey's multiple comparison tests, significance refers to $P \leq 0.05$. Properties of organic materials were compared by t test. Statistical analysis was carried out in IBM SPSS Statistics 24.

3. Results

Sandy soil alone had the following properties: 98% sand, 1% silt, 1% clay, $EC_{1:5}$ 14.3 µS cm^{-1}, $pH_{1:5}$ 6.3, WHC 0.008 g water g^{-1} soil, TOC 0.18 g kg^{-1}, available N 11.93 mg kg^{-1} and available P 0.36 mg kg^{-1}. In W, total organic N was almost three times higher than in CM (Table 1). But total N and P in CM were more than three times higher than in W. Therefore, total N and P added increased with increasing proportion of CM in the mix, whereas total organic C added decreased (Table 2).

Table 1. Selected properties of organic materials used (n=5, mean ± standard error). Within column, means followed by different letters are significantly different ($P \leq 0.05$).

Organic materials	Total C	Total N	Total P	C/N	C/P
	g kg^{-1}				
Cow manure	136.1 a ± 9.4	19.7 a ± 0.9	5.6 a ± 0.1	7 a	24 a
Wheat straw	391.2 b ± 24.5	5.5 b ± 0.4	1.7 b ± 0.1	71 b	228 b

Table 2. Total C, N and P added in treatments.

Treatment	Total C (g C kg^{-1} mix)	Total N (g N kg^{-1} mix)	Total P (g P kg^{-1} mix)
100W	3.91	0.05	0.02
75W-25CM	3.27	0.09	0.03
50W-50CM	2.64	0.13	0.04
25W-75CM	2.00	0.16	0.05
100CM	1.36	0.20	0.06

3.1 Cumulative respiration

Compared to the unamended control, cumulative respiration per g soil was highest in 100W where it was 12 fold higher than the control (Figure 1). Cumulative respiration in 100CM was similar as in the control.

Cumulative respiration per g C added was higher in mixes with 50% or more W than in mixes with lower proportion of W (Figure 1).

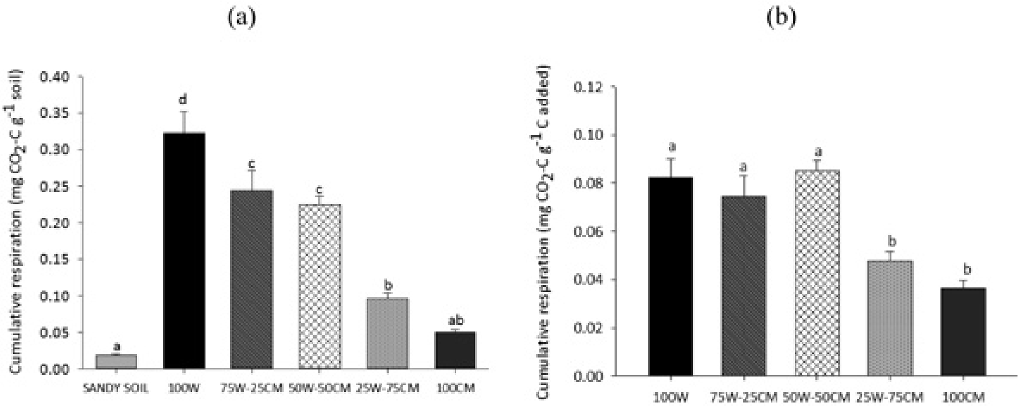


Figure 1. Cumulative respiration after 25 days in mg CO_2-C g^{-1} soil (a) and mg CO_2-C g^{-1} C added (b) in sandy soil alone or amended with wheat straw or cow manure only and both at different ratios. Bars with different letters are significantly different (n=5, $P \leq 0.05$).

3.2. Available N and P and microbial biomass before the 1st and after 2nd leaching

In amended soils, available N and P per kg soil were higher before the 1st leaching than after the 2nd leaching (Table 3). On day 9, available N in amended soils was higher than in the un-amended control except for 50W-50CM and 100CM, however there was no significant difference among treatments on day 26.

Table 3. Available N, available P and microbial biomass N and P (mg kg^{-1} soil) in soil before 1st (day 9) and after the 2nd leaching event (day 26) in sandy soil alone or amended with wheat straw or cow manure only and both at different ratios. On a given day, values with different letters are significantly different (n=5, $P \leq 0.05$**).**

Treatment	**Available N**		**Available P**		**MBN**		**MBP**	
	mg kg⁻¹ soil							
	Day 9	Day 26	Day 9	Day 26	Day 9	Day 26	Day 9	Day 26
Control	2.94 a	2.93 a	0.13 a	0.40 a	0.09 a	0.23 a	0.03 a	0.05 a
100W	5.10 b	3.94 a	0.77 a	0.56 a	5.47 b	0.78 a	1.61 ab	0.11 a
75W-25CM	5.47 b	3.75 a	3.71 b	2.32 b	2.41 a	0.03 a	2.85 ab	1.40 ab
50W-50CM	4.63 ab	3.29 a	8.11 c	5.21 c	2.39 a	0.64 a	2.06 ab	0.46 a
25W-75CM	5.47 b	2.52 a	11.05 d	6.87 d	0.81 a	0.02 a	2.75 ab	0.57 a
100CM	3.93 ab	2.15 a	16.54 e	7.62 d	0.72 a	0.44 a	3.55 b	3.45 b

Available P per kg soil on days 9 and 26 in amended soils was higher than that the control except for 100W. It increased with proportion of CM in mixes (Table 3). Available P was highest in 100CM on day 9 but similar in 25W-75CM and 100CM on day 26.

MBN per kg soil on day 9 was highest in 100W where it was 50 fold higher than in the control, but there was no significant difference among treatments on day 26 (Table 3). MBP on days 9 and 26 was higher than the control only in 100CM.

Available N per g N added reflected an opposite pattern than available P per g P added (Figure 2). Available N per g N added was highest in 100W where it was about five-fold higher than in 100CM (Figure 2). It decreased with proportion of W in the mix until 50W-50CM. There was little difference in available N between treatments with 50% or more percent CM. Differences among treatments were greater on day 9 than day 26.

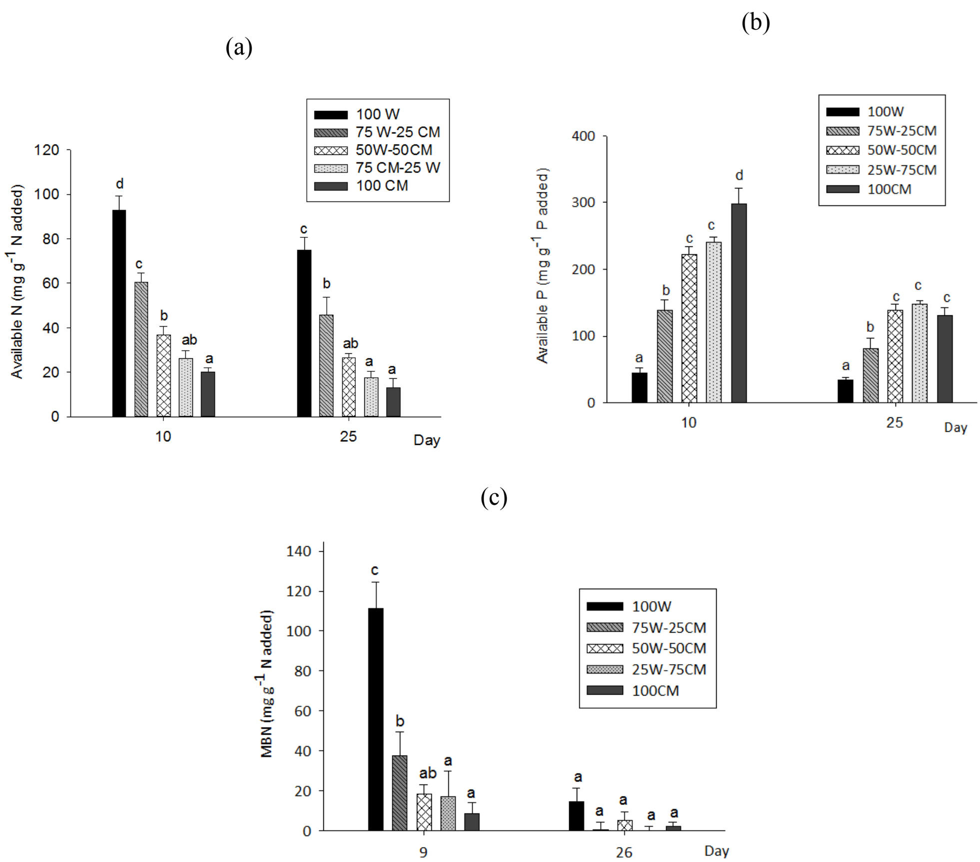


Figure 2. Available N per g N added (a), available P per g P added (b), MBN per g N added (c), before 1st leaching (day 9) and after the 2nd leaching (day 26) in sandy soil amended with wheat straw or cow manure only and both at different ratios. At a given sampling time, bars with different letters are significantly different (n=5, $P \leq 0.05$).

Available P per g P added on day 9 was highest in 100CM where it was more than six-fold higher than in 100W, followed by 25W-75CM and 50W-50CM (Figure 2). Available P per g P added in 75W-25CM was only a half of that in 100CM. On day 26 after the 2nd leaching, available P per g P added was lower in 75W-25CM and 100W than in 100CM, however there was no significant difference in mixes with ≥ 50% CM.

MBN per g N added on day 9 was highest in 100W where it was 12 times higher than in 100CM followed by 75W-25CM where it was about a third of that in 100W (Figure 2). MBN per g N added did not differ among treatments with ≤ 50% W. On day 26, MBN per g N added was very low and did not differ among treatments (Figure 2).

MBP per g P added in did not differ among treatments for both leaching events (Table 4).

Table 4. MBP per g P added before 1st (day 9) and after the 2nd leaching event (day 26) in sandy soil amended with wheat straw or cow manure only and both at different ratios. On a given day, values with different letters are significantly different (n=5, $P \leq 0.05$)**.**

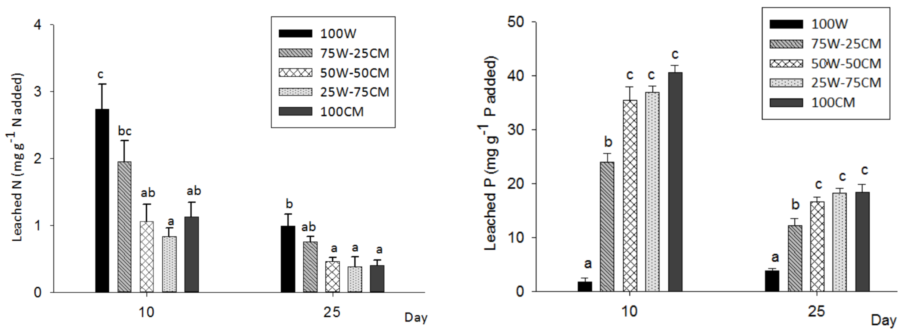
Treatment	**MBP**	
	mg g^{-1} P added	
	Day 10	Day 25
100W	93.9 a	6.4 a
75W-25CM	106.7 a	52.3 a
50W-50CM	56.6 a	12.7 a
25W-75CM	59.8 a	12.4 a
100CM	63.8 a	61.97 a

3.3 Inorganic N and P in leachate of both leaching events

Leachate nutrient concentration was lower in the 2nd leaching compared to the 1st (Table 5, Figure 3). For both leaching events, leached inorganic N per kg soil was low and did not differ among treatments (Table 4). Leached inorganic P on days 10 and 25 was similar in the control and 100W, where it was up to 40-fold lower than in the other treatments (Table 4). In amended treatments with ≤75% W, inorganic P in leachate increased with proportion of CM and was about twice as high on day 10 than day 25. WEOC in leachate per kg soil on day 10 was lowest in the control and highest in 100W (Table 4). It decreased with proportion of W up to 50W-50CM, but was similar in treatments with ≥ 50% CM. WEOC per kg soil decreased up to four-fold in amended soils from day 10 to day 25. On day 25, WEOC was higher than the control only in 100W and 75W-25CM.

Table 5. Inorganic N and P and water-extractable organic C (WEOC) of leachate (mg kg^{-1} soil) of the 1st and 2nd leaching in sandy soil alone or amended with wheat straw or cow manure only and both at different ratios. On a given day, values with different letters are significantly different (n=5, $P \leq 0.05$).

Treatment	Inorganic N		Inorganic P		WEOC	
	mg kg^{-1} soil					
	Day 10	Day 25	Day 10	Day 25	Day 10	Day 25
Control	0.15 a	0.07 a	0.06 a	0.04 a	10.89 a	9.20 a
100W	0.17 a	0.05 a	0.03 a	0.07 a	90.65 d	17.84 c
75W-25CM	0.18 a	0.07 a	0.64 b	0.33 b	73.94 c	14.03 b
50W-50CM	0.13 a	0.06 a	1.29 c	0.60 c	56.23 b	12.35 ab
25W-75CM	0.13 a	0.06 a	1.70 d	0.84 d	50.82 b	11.79 ab
100CM	0.22 a	0.08 a	2.26 e	1.02 e	46.40 b	11.82 ab



(c)

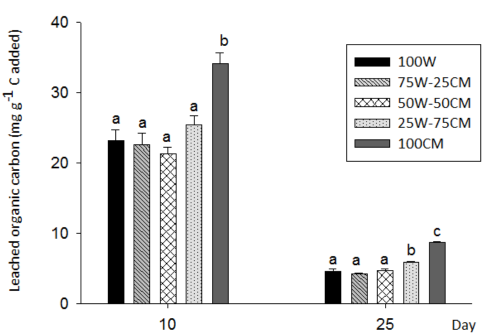


Figure 3. Leached inorganic N per g N added (a), inorganic P per g P added (b) and organic C per g C added (c) in the 1st (day 10) and the 2nd leaching event (day 25) in sandy soil amended with wheat straw or cow manure only and both at different ratios. At a given sampling time, columns with different letters are significantly different (n=5, $P \leq 0.05$).

Leached N per g N added was higher in 100W than in treatments with ≤ 50% W (Figure 3). It was about two to three-fold higher in the first than the second leaching event. Compared to 100W, leached P in 100CM was 12-fold higher on day 10 and five-fold higher on day 25. In both leaching events, leached P per g P added was higher in amendments with ≥50 % CM than those with lower proportion of CM (Figure 3).

On days 10 and 25, leached organic carbon per g C added was highest with 100CM where it was ≥ 30% higher than in the other treatments (Figure 3). Leached organic C per g C added was about four-fold lower on day 25 than day 9.

3.4 Measured compared to expected values

For both leaching events, measured available N, leached N and MBN per g N added and leached organic C per g C added were lower than expected values (Figure 4). Measured values were 20-40% lower for available N, 40-98% for MBN, 10-50% and 20-30% for leached N and organic C, respectively.

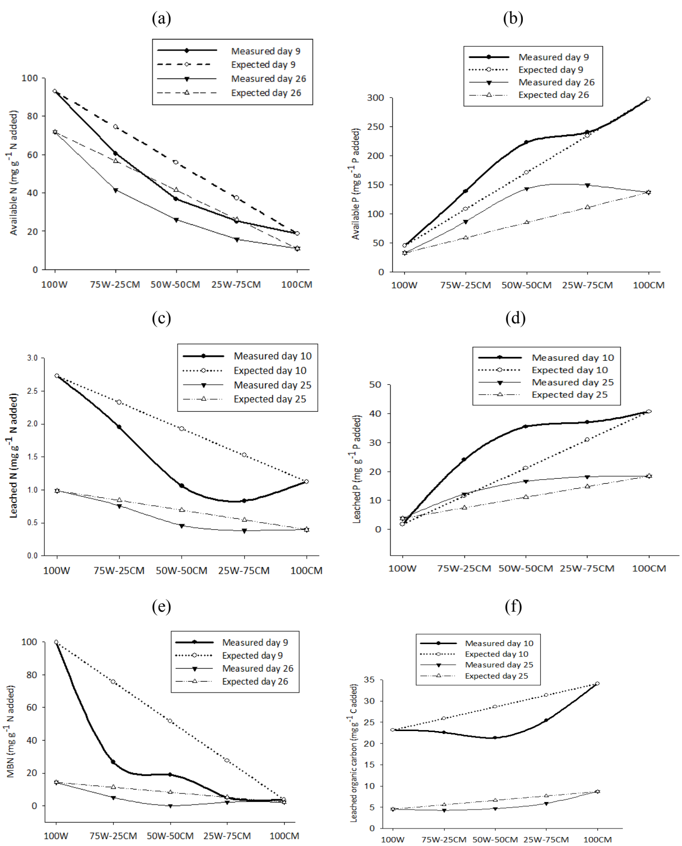


Figure 4. Expected and measured available N (a), available P (b) before 1st leaching (day 9) and after the 2nd leaching (day 26), and leached inorganic N (c), leached inorganic P (d), MBN (e), leached organic C (f) in the 1st (day 10) and the 2nd leaching event (day 25) in sandy soil amended with wheat straw or cow manure only and both at different ratios expressed in mg per g C, N and P added.

In contrast, measured available P and leached P per g P added were higher than the expected values for both leaching events (Figure 4). Measured available P and measured leached P were 2-70% and 20-100% higher than expected values, respectively.

3. Discussion

Based on this study, the first hypothesis (due its low N and P concentration compared to cow manure, addition of wheat straw will result in lower N and P availability and N and P in leachate than with cow manure) has to be declined, but the second hypothesis (in mixes, non-additive interactions will dominate, but depend on parameter assessed) can be confirmed..

3.1. Comparison 100W and 100CM

Wheat straw is generally considered to be slowly decomposable because of its high C/N and C/P ratio and high proportion of structural carbohydrates (Alexander, 1977; Xiao *et al.*, 2001). But in this study, CM was less decomposable than W despite its high N and P content. Manure is the result of microbial decomposition in the gut of animals and may be further decomposed during storage. Thus, it contains very little rapidly decomposable compounds and has a low C/N and C/P ratio. The highly decomposed state of the manure explains why addition of CM did not increase cumulative respiration compared to the unamended control. This also explains the low organic C concentration per kg soil in the leachate in 100CM compared to 100W. CM contained three-fold more total N and P than W, but when expressed per kg of soil, available N and leached inorganic N was similar in 100CM and 100W and MBN on day 9 was lower in 100CM than 100W. On the other hand, available P and leached P were 10 and 15-fold higher in 100CM than 100W. Further, MBP on day 26 was 26-times higher in 100CM than 100W. These differences in nutrient release between the two amendments were also evident when available and leached N and P are expressed per g N and P added. The low N availability and leached N in 100 CM suggests that N in CM is in slowly decomposable compounds such as lignin. In contrast, a large proportion of P in CM appears to be in water-soluble and rapidly decomposable forms. Manures have been shown to contain large amounts of P when animals are fed a P-rich diet (Morse *et al.*, 1992). Per g C added, leached organic C was higher in 100 CM than 100W, whereas cumulative respiration was lower. This suggests that compounds in leached organic C of 100CM were slowly decomposable, e.g. aromatic compounds.

3.2 Mixes

To better understand nutrient dynamics in mixes, the following discussion is about available and leached N and P and MBN, MBP per g N and P added. As expected from the data of 100W and 100CM, available N, MBN and leached N decreased with proportion of wheat straw whereas available and leached P increased. However, the decrease or increase were not linear as the comparison between measured and expected values shows. The lower than expected available and leached N and MBN suggests that presence of CM inhibits mineralisation of organic N in wheat straw, possibly through aromatic compounds that either inhibit microbes or form stable compounds with proteins (Lucchini *et al.*, 1990). The higher than expected available and leached P indicates that presence of W enhanced release of P from CM. Increased release could be due to changed physical environment (e.g. better accessibility of CM particles). Another possible reason is that microbes decomposing W stimulated organic P mineralisation in CM to satisfy their P demand.

4. Conclusions

This study showed that addition of cow manure to soil may not result in high N leaching despite its low C/N ratio. The study further showed that mixing of cow manure and wheat straw can be used to reduce N leaching but may enhance P leaching. The high P availability and P leaching potential of cow manure could be beneficial for crops in P deficient soils, but may also have negative effects by increasing eutrophication. We used sand to minimize sorption of nutrients to soil particles. In soils with higher silt and clay content, N and P leaching is likely to be lower than in this study because released N and P are bound to clay minerals as well as organic matter.

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