Effect of treated and untreated farm dairy effluents on soil fertility microbial population growth, plant growth, and plant chemical composition

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Qiushi Du

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The expansion of the dairy farming industry has resulted in a large amount of farm dairy effluent (FDE) being produced. Land application of FDE is used to recycle the nutrients in the FDE. ClearTech® is a new effluent treatment technology desingned to separate the solids from the liquids and thus produce treated effluent (TE) and clarified water (CW). The CW is recycled as wash water for the farm yard, while the TE is applied to land to recycle the nutrients. However, the effect of how the treated effluent on soil fertility indices, microbial population growth, plant growth, and plant chemical composition compared with land application of untreated effluent (UE) are largely unknown.

Thus, the objectives of this research were: a) to determine the effects of treated and untreated FDE on soil fertility indices; b) to determine the abundance of ammonia-oxidising bacteria (AOB), ammonia-oxidising archaea (AOA), denitrifying functional genes (nirS, nirK and nosZ), general agrobacteria (16S rRNA) and fungi (18S rRNA) following the application of treated and untreated of FDE; and c) to determine the effects of the treated and untreated FDE on plant yield and plant chemical composition.

A field experiment was conducted to measure key soil properties, the abundance of AOB, AOA, denitrifying functional genes (nirS, nirK and nosZ), plant yield, and the nutrient concentrations in the pasture. The trial was located at the Lincoln University Research Dairy Farm on a Templeton silt loam soil. FDE was collected from the Lincoln University Demonstration Dairy Farm. FDE was treated to produce treated effluent (TE) and clarified water (CW) by the ClearTech® treatment technology. The TE, the original untreated effluent (UE) and water (control) were applied to the soil plots. Soil samples were taken after 1 and 14 days, and 1, 2 and 3 months following each treatment application, and the pasture was harvested following typical grazing schedules.

Results showed that the content of soil organic matter, total C, total N and Olsen P and the abundance of denitrifying functional genes were higher after the application of TE than UE. There were no significant differences between TE and UE in mineral N dynamics, CEC, the abundance of AOB, AOA, general agrobacteria and fungi, plant yield and the plant chemical composition. Therefore, it is concluded that the application of the TE produced from the ClearTech® treatment technology will result in higher the contents of soil organic matter, total C, total N and Olsen P and the abundance of denitrifying functional genes compared with the UE whilst mineral N dynamics, CEC, the abundance of AOB, AOA, general agrobacteria and fungi, plant yield and the plant chemical composition will be similar. Future research could assess potential effects of long-term applications of the TE, the effect of climatic conditions and different soil types on the soil properties and plant growth arising from the application of the different effluents.

Keywords: farm dairy effluent, effluent treatment technology, treated effluent, soil fertility, organic matter, ammonium, nitrate, ammonia oxidising bacteria, ammonia oxidising archaea, denitrifiers, plant yield, plant chemical composition, ClearTech®.

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Chapter 1

Introduction

1.1 Introduction

Dairy production is a major export earner for New Zealand as it contributes a quarter of New Zealand's export earnings (Foote et al., 2015). In the past two decades, the expansion of New Zealand dairy farming has been dramatic. Data from DairyNZ (2018) shows that the population of dairy cattle rose from around 3 million in 1996/97 to nearly 5 million in 2016/17. The North Island has over two-thirds of the total dairy herds (72.6%) which are mainly concentrated in the Waikato region (28.8%). Although only 27.4% of the national total is located in the South Island, they account for 40.4% of the total number of cows (DairyNZ, 2018). Figure 1.1 shows the regional distribution of dairy cows in 2016/17 among which 23% of dairy cows are in the Waikato region, ahead of North Canterbury (13.8%) and Southland (11.6%). Although the intensification of dairy farming has resulted in the growth of milk production, serious environmental pollution has followed, including water contamination and greenhouse gas emissions (Di & Cameron, 2016; Foote et al., 2015).

Figure 1.1 Regional distribution of dairy cows in 2016/17 (DairyNZ, 2018).

Irrigation of farm dairy effluent (FDE) is now the norm in New Zealand and the main recycling method used worldwide (Müller et al., 2007). FDE is the mixture of dairy cow excreta, water, cleaning fluids, and milk which is produced during the cleaning of the holding yards and milking equipment (Hawke & Summers, 2006). Generally, FDE contains only 10% excreta and 90% wash-water plus other material (Longhurst et al., 2000). The high water content and nutrient content of FDE made it recyclable for irrigation. The benefits of FDE irrigation include providing nutrients for plant development, improving soil structure, and increasing pasture yield since animal excreta contain significant amounts of nutrients (Müller et al., 2007). Animal urine and faeces are the major sources of nitrogen (N) in FDE as most of the N consumed by dairy cows is returned to pasture in excreta (Di & Cameron, 2016). Besides, there are also various other valuable nutrients, such as phosphorus (P), potassium (K), and quantities of trace elements in the FDE (Wang et al., 2004; Luo et al., 2008). However, there are also several potential disadvantages and risks from the improper management of FDE. These consist of excess amounts of nutrients (N and P) causing eutrophication if they get into waterways (Wang et al., 2004; Müller et al., 2007). The odour from the application of FDE is also a public issue (Wang et al., 2004).

Recently, a new technology for FDE treatment called ClearTech® has been developed. Unlike previous methods, it can separate the liquid and solid components to reuse the water to wash the yard, reduce the amount of effluent needing to be stored on the farm, and recycle the nutrients effectively (Cameron & Di, 2018). However, the different effects of treated and untreated farm dairy effluent on soil fertility, microbial population growth, plant growth, and plant chemical composition are still unknown. This research programme is designed to answer some of these questions.

1.2 Aims and objects

The aims of this study were to improve knowledge and fundamental understanding of the effect of applying different forms of FDE (including untreated standard FDE, clarified water and treated effluent) on soil fertility indices, soil microbial population growth, and plant growth.

The objectives of this project were:

a) To determine the effects of treated and untreated FDE on soil fertility indices;

b) To determine the abundance of ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), denitrifying functional genes (*nir*S, *nir*K, and *nos*Z), general agrobacteria (16S rRNA) and fungi (18S rRNA) following the application of different forms of FDE;

c) To determine the effects of the treated and untreated FDE on plant yield and plant chemical composition.

1.3 Hypotheses

This research programme will test the following hypothesis:

That ClearTech® treated farm dairy effluent will have similar effects on key soil fertility indices, soil microbial population growth, plant growth, and plant chemical composition as untreated standard farm dairy effluent.

1.4 Structure of the thesis

Chapter Two of this thesis provides a review of previously published literature relevant to FDE and different types of FDE management systems. The experimental design and methods of sampling and analysis in the research are described in Chapter Three. Chapter Four presents the research results and discussion. Chapter Five summarizes the conclusion of this research and provides some suggestions for future research.

Chapter 2

Literature Review

2.1 Introduciton

In New Zealand grazing systems, the majority of cow excreta (urine and faeces) are deposited on the pasture during grazing, however the excreta deposited in the milking shed has to be managed as farm dairy effluent (FDE) (Chung et al., 2013; Laubach et al., 2015). FDE is a mixture of cow urine and faeces diluted by wash-down water, detergents, acids, and other cleaners.

Generally, FDE is comprised of about 10% cow excreta and 90% wash-water (Gibson, 1995). It also contains a variety of valuable nutrients for plant growth such as nitrogen (N), phosphorus (P), organic carbon (C), potassium (K) and, sulphur (S) (Hawke & Summers, 2006; Li et al., 2014). Annually, each dairy cow can generate excreta containing 5.9 kg N, 0.7 kg P, 5.4 kg K. 0.8 kg S, 2.2 kg Ca, 1 kg Mg, and 0.7 kg Na (Heatley, 1995). Previous research showed that the compositional variations of FDE were dependent on the feed types, cow's age and breed, milking time, fertiliser conditions and feed quality (Cooke et al., 1979; Goold, 1980; Longhurst et al., 2000; Hawke & Summers, 2006).

With the intensification of the dairy industry, an increasing amount of FDE in New Zealand has been generated. However, inadequate management of FDE may result in serious environmental issues, including the negative effects on the quality of surface water, the risks of high nitrate content in groundwater, and the problem of odour (Longhurst et al., 2000; Ali et al., 2006). In addition, the huge volumes of water used by agriculture and the low water use efficiency also cause significant environmental and resources problems. Cameron and Di (2018) indicated that a New Zealand farm with c. 400 cows needed an average of 28,000L wash-down water per day.

The irrigation of FDE has been widely used in New Zealand dairy industry since last century as it can improve the soil fertility and increase pasture growth (Cameron et al., 2014; Cameron & Di, 2018). There are several FDE management systems used in New Zealand such as two-pond systems, direct land irrigation, deferred effluent irrigation, and ClearTech®. This review will first provide a brief summary of the nutrient value in FDE, followed by a review of the benefits and risks of FDE application, and the description of different types of FDE management systems.

2.2 Nutrient values of FDE

2.2.1 Solids and Water Content

FDE is a very diluted organic effluent containing less than 1% solids content and 99% of water (Barkle et al., 2001; Hawke & Summers, 2006). However, the actual content is determined by the amount of wash-water used on the farm and the size of the average dairy herd (Longhurst et al., 2000). The study of Longhurst et al. (1999) summarized the solid content of 63 sites over 20 years and reported an average of 0.9% solids (Table 2.1).

Table 2.1 Summary of solids content (% dry matter) found in farm dairy effluent (Longhurst et al., 1999).

Sites	Mean	SD	Lowest	Highest	Source
1	0.72	0.18	0.50	1.20	Warburton (1977)
1	0.50	-	0.08	2.70	MacGregor et al. (1979)
4	0.68	0.28	0.28	0.93	Taranaki Regional Council (1990)
40	0.82	0.91	0.91	4.96	Longhurst et al. (1999) (sumps)
8	1.36	1.73	1.73	5.20	Longhurst et al. (1999) (sumps)
8	1.27	0.68	0.68	2.23	Longhurst et al. (1999) (irrigators)
1	0.92	0.44	0.44	1.94	Longhurst et al. (1999) (sump)
Mean (n=63)	0.90	-	-	-	

2.2.2 Nitrogen

N is an essential element for the growth of plants and is also the main constituent of amino acids, chlorophyll and various enzymes and co-enzymes (McLaren & Cameron, 1996). Since N is an important component of many substances in plants, there is a great demand for N in plant growth.

In nature, various N forms can be found in both the earth's crust and the atmosphere (N_2). However, only certain forms of N can be directly absorbed by plants. Nitrate (NO_3^-) and ammonium (NH_4^+) are the two available mineral forms which plant can take up. The N cycle shows the transfer of different N forms among the atmosphere, soil, plants and animals (Figure 2.1).

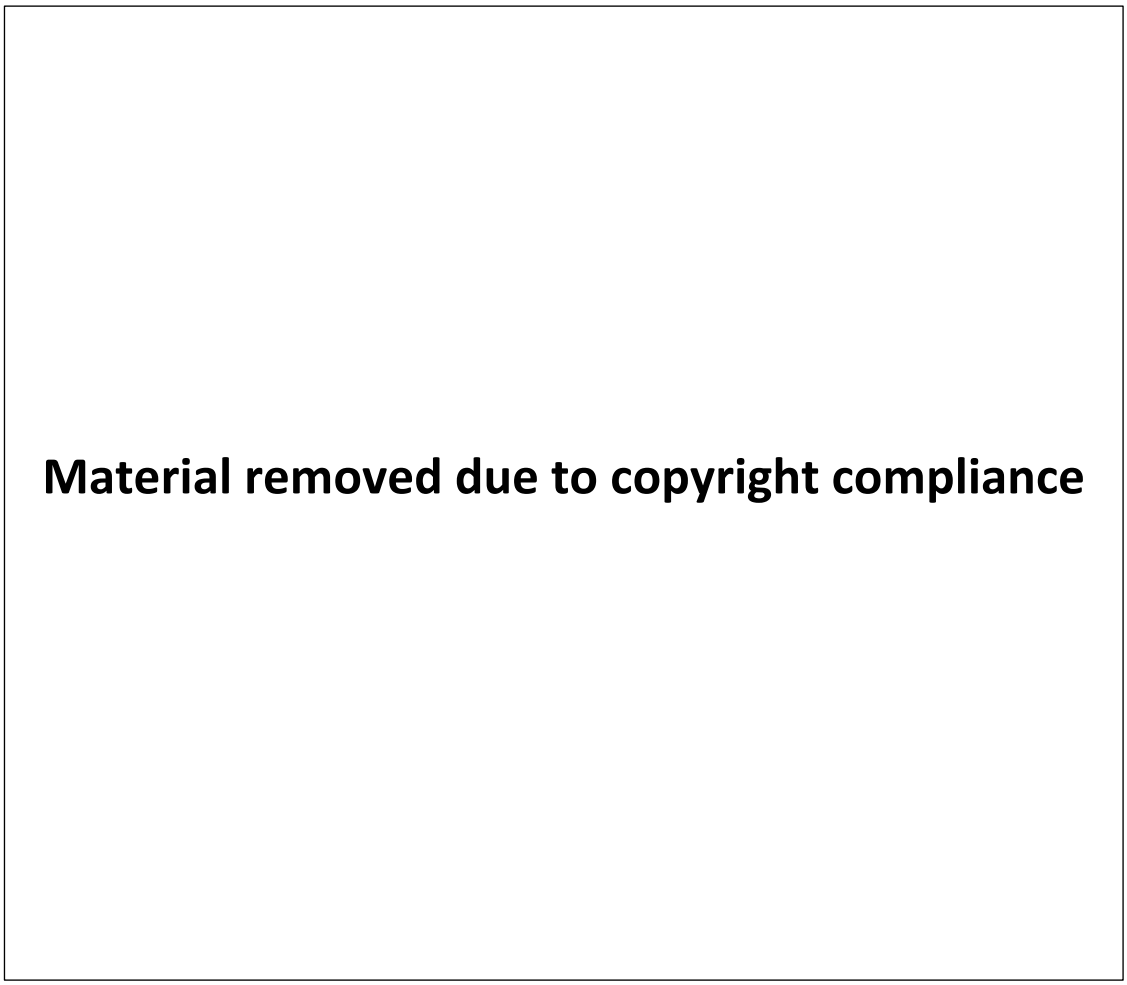


Figure 2.1 The nitrogen cycle (McLaren & Cameron, 1996).

The forms of N returned by grazing animals are urea from urine and organic N from faeces (Hawke & Summers, 2006). In FDE, organic N usually accounts for 60-85% of total N (Selvarajah, 1996; Barkle et al., 2001; Hawke & Summers, 2006). Although urine also contains some organic compounds, the major N form of urine-N is urea (60-90%) which can be hydrolysed to ammonium (Hawke & Summers, 2006). The hydrolytic process can be expressed as (1.1):

$$(NH_2)_2CO + 2H_2O \rightarrow (NH_2)_2CO_3 \leftrightarrow NH_4^+ + NH_3 + CO_2 + OH^- \quad (1.1)$$

The amino compounds are converted into ammonia due to the participation of soil microorganisms (1.2).

$$R\text{-}NH_2 + H_2O \rightarrow NH_3 + R\text{-}OH + energy \quad (1.2)$$

The nitrification process converts ammonia (1.3 and 1.4) into nitrite followed by transformation of nitrite (NO_2^-) into nitrate (NO_3^-).

$$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + \text{energy} \quad (1.3)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{energy} \quad (1.4)$$

Compared with urea and organic N, researchers report there is only a small amount of nitrate in the FDE (Table 2.2).

Table 2.2 The content of different forms of N in FDE, including total-N, ammonium-N, nitrate-N, and organic N.

Forms of N In FDE	Total N (mg/L)	Ammonium-N (mg/L)	Nitrate-N (mg/L)	Organic N (mg/L)	Source
	167/198	13.5/26	2.2/2/3		Cooke et al. (1979)
	195(56)				Goold (1980)
	363(199)	95(49)	0.5(0.6)		Di et al. (1998)
	240	61	0.19		Silva et al. (1999)
	269(181-506)	48(13-132)	2(1-6)	219(144-374)	Longhurst et al. (2000)
	99(44-186)	23(5-70)	0.05(<0.05-0.45)		Singleton et al. (2001)

2.2.3 Phosphorous

P is an important component of many compounds in plants, such as adenosine triphosphate (ATP), adenosine diphosphate (ADP) and the nucleic acids (McLaren & Cameron, 1996). ATP is the major source of energy in the plant produced by photosynthesis and metabolism. The transfer of energy in the plant is accomplished by converting ATP into ADP by releasing P. Then, the energy can be stored by the process of ADP binding to phosphate groups to form ATP. P is also the key component to connect nucleic acids including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) which determine the development of the plant. Typically, plant dry matter contains 0.1-0.5% P, and the P content in the pasture is between 0.3 and 0.4 percent of dry matter (McLaren & Cameron, 1996). Plants mainly absorb $H_2PO_4^-$ and HPO_4^{2-} ions from the soil solution. The P cycle in a grazed pasture system is shown in Figure 2.2.

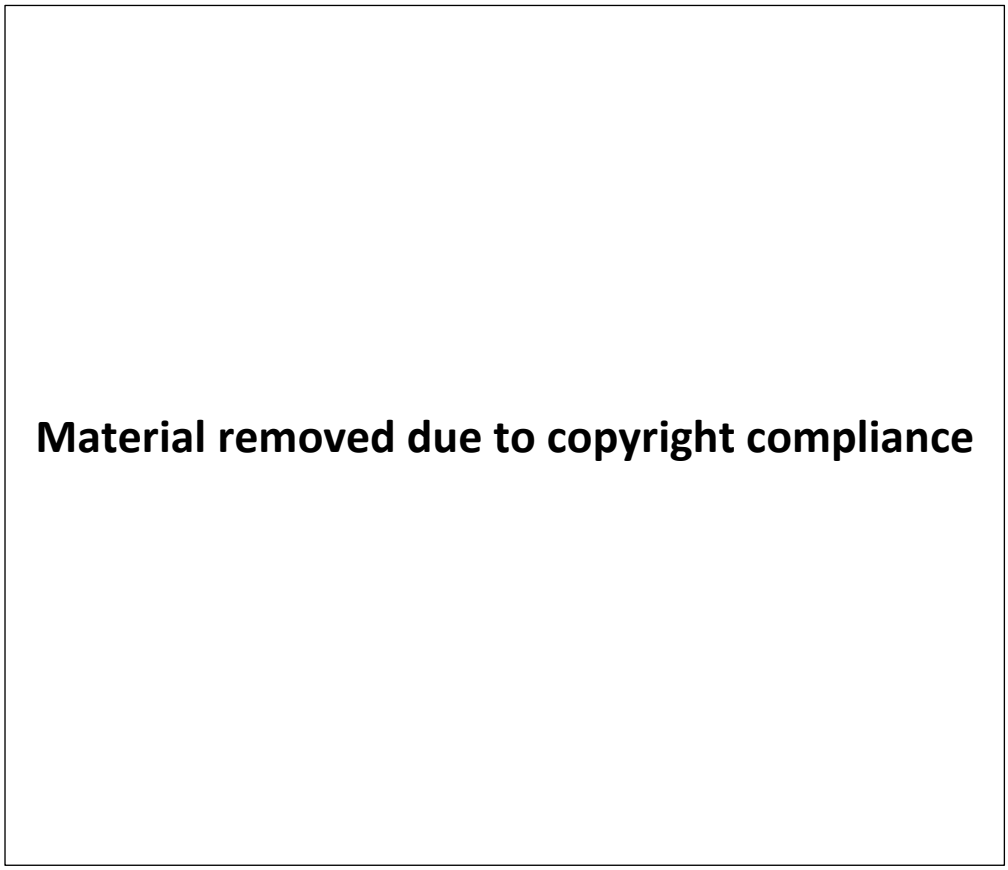


Figure 2.2 The phosphorus cycle in a grazed pasture system (McLaren & Cameron, 1996).

Dairy cows are the major contributors to the P cycle in grazed pastures. Inorganic P from dung can be absorbed by the plant (Aarons et al., 2004). Toor et al. (2004) also indicated that 86% of the total P in FDE was inorganic forms while organic P was less than 10%. Table 2.3 summarized the contents of total P and phosphate in FDE from previous studies.

Table 2.3 The content of P in FDE (g m^{-3}).

Component	No. of samples	Mean	Range	Reference
Total-P	73	69	21-82	Longhurst et al. (2000)
	8	55	23-123	Di and Cameron (2002)
	6	31		Hawke and Summers (2003)
		22		Bolan et al. (2004)
PO_4^- -P	5	40	22-61	Di et al. (1998)
	6	15		Hawke and Summers (2003)

2.2.4 Other elements

FDE also contains other nutrient elements such as S, K, Ca, Na and Mg which are essential nutrients for plant growth. Nearly 90% of the total S in plants exists in the amino acids cysteine and methionine which are constituents of protein (McLaren & Cameron, 1996). S is also a component of coenzyme-A and vitamins. K is not a compound of the plant fabric, but it controls the regulation of stomatal opening related to transpiration and photosynthesis. Besides, K is also essential for balancing the negative charge of anions, the activation of many enzyme systems, and the synthesis of protein and starch. Ca is important for the growth of root tips and the cell wall. Mg is an important component of the chlorophyll molecule which plays a decisive role in the photosynthesis process.

The concentrations of those major elements are reported in Table 2.4 Among them, the concentrations of K are relatively high. Longhurst et al. (2000) reported that K concentrations achieved a value of 370 g m^{-3} while the concentrations of P were only 70 g m^{-3}. In addition, many studies have shown that the content of exchangeable K, Na, Ca and Mg of soil increases after long-term irrigation of farm effluent (Hawke & Summers, 2006).

Table 2.4 The content of different nutrients in FDE (g m^{-3}).

Components	No. of samples	Mean	Range	Reference
Total S	41	65	52-65	Longhurst et al. (2000)
SO_4-S	5	0.5	4-19	Di et al. (1998)
K	58	370	164-705	Longhurst et al. (2000)
	6	53		Hawke and Summers (2003)
		231		Bolan et al. (2004)
Na	6	19		Hawke and Summers (2003)
Ca	6	33		Hawke and Summers (2003)
		15		Bolan et al. (2004)
Mg	6	15		Hawke and Summers (2003)
		12		Bolan et al. (2004)

2.3 Application of Farm Dairy Effluent

The intensification of dairy farming has contributed to rapidly increased volumes of cattle excreta being generated. Nearly 77% of FDE is now collected in ponds for recycling (Laubach et al., 2015). However, inadequate treatment of FDE and poor irrigation management of FDE has caused a decline in water quality via the leaching and runoff of nutrients, faecal microorganisms, and sediment (Longhurst et al., 2000; Laurenson et al., 2017).

Application of FDE to grazing farms is now recognized as the preferred method to treat FDE (Cameron et al., 1997; Degens et al., 2000). FDE application has both positive and negative effects on the environment and human health (Xu et al., 2010). As FDE contains a large amount of diverse nutrients, it can improve the soil quality and increase the productivity of pastures and animals (Longhurst et al., 2000; Sparling et al., 2015). However, the application of FDE with high pH and sodium adsorption may lead to the dissolution of organic matter and nutrients from the soil (Degens et al., 2000). The loss of organic matter can result in a change of soil structure and loss of nutrient retention and water-holding capacity (Doran & Parkin, 1994; Carter et al., 1997; Degens et al., 2000). FDE application may cause a large amount of N leaching especially from well-structured soils and wet soils. Greenhouses gases emissions from FDE collection ponds and land application areas are also large contributors to GHG emissions in New Zealand (Laubach et al., 2015). Wang et al. (2004) summarized the beneficial and adverse effects of the FDE application as shown in Table 2.5.

Table 2.5 Beneficial and adverse effects of land application of farm effluent (Wang et al., 2004).

Effects	Benefits	Potential hazards
Description	Providing a source of irrigation water and nutrients of plants	Nitrate leaching to groundwater Phosphorus loss to waterways
	Improving soil fertility and productivity	Heavy metal accumulation in soil
	Reducing direct contaminant discharge to surface water	Enhancing organic contaminant mobility in soil Odour and gaseous emission Inducing nutritional disorder of animals Pathogen-related health issues Oestrogen entering waterways

2.4 Types of FDE management systems

2.4.1 Two-pond systems

In New Zealand, two-pond systems have been used as the traditional method for FDE treatment since the 1970s (Laubach et al., 2015). This practice is divided into two parts: anaerobic conditions in the first pond and aerobic conditions in the second (Figure 2.3). In the first pond, anaerobic fermentation can digest the organic matter in FDE, then digested faeces and soil are separated out and sink to the bottom of the pond. The second pond is much larger and shallower as the top layer can be used for aerobic treatment while the bottom can continue providing an anaerobic treatment (Houlbrooke et al., 2004). This system can effectively reduce biological oxygen demand, chemical oxygen demand and total suspended solids (Bolan et al., 2004; Craggs et al., 2004).

Figure 2.3 Schematic diagram of a dairy farm oxidation pond system, and anaerobic pond, followed by a facultative pond (Craggs et al., 2004).

However, the discharge of FDE after the treatment can also have negative impacts on a water body. As nutrients including N and P cannot be removed from the effluent, the high nutrient levels may lead to eutrophication and the propagation of nuisance plants if the FDE is leached into waterways (Bolan et al., 2004; Craggs et al., 2004; Houlbrooke et al., 2004; Wang et al., 2004). High concentrations of ammoniacal-N may cause an increase in pH and be toxic to aquatic life (Craggs et al., 2004). In addition, the treated effluent also contains high concentrations of faecal bacteria and algal solids. The high content of faecal bacteria indicates the potential risks to drinking water which may influence the health of people and livestock. The algal solids may obstruct the respiration of the bed stream, resulting in anaerobic conditions (Quinn & Hickey, 1993;Craggs et al., 2004). Two-pond systems have been phased out in New Zealand from the 1990s (Houlbrooke et al., 2004).

2.4.2 Direct land irrigation

Land application of FDE, from two-pond systems or directly from the daily wash-down water, is now the most popular method to manage FDE in New Zealand (Houlbrooke et al., 2004). Direct land irrigation has become a preferred treatment method from the mid-1990s (Laubach et al., 2015). This system uses traveling irrigators to irrigate the FDE from a small sump. The sump is generally located at the cowshed and cannot store the FDE in it, thus, FDE needs to be applied daily, or transfer to a holding pond. The simulation of Houlbrooke et al. (2004) shows the volume of FDE and nutrients lost under different irrigators (Table 2.6). On average, the direct drainage loss can represent 14% of the total annual volume of the applied FDE. When the application depth is lower than 30 mm, the losses of applied FDE decrease.

Land application of FDE still has the risk of nutrient losses. Soil saturation under wet weather and failure of irrigation equipment may lead to FDE flowing from the pasture into waterways. Daily irrigation can also cause nutrient leaching from the pasture root zone into groundwater. In addition, the amount of nutrients released is also affected by the depth of the FDE application.

Table 2.6 The predicted direct drainage loss of farm-dairy effluent (FDE) volume and nutrients under a range of different irrigator and soil moisture scenarios for an average application depth of 25 mm, under relatively calm wind conditions (Houlbrooke et al., 2004).

Irrigator Scenario	Soil moisture deficit (mm)	% of applied FDE that drains	Predicted drainage loss of N (kg ha^{-1})	Predicted drainage loss of P (kg ha^{-1})
Rotating irrigator	18	29	4	0.62
	25	14	1.9	0.29
	32	6	0.8	0.12
Oscillating irrigator	18	30	4.1	0.64
	25	7	1	0.16
	32	0	0.0	0.0

2.4.3 Deferred effluent irrigation

The deferred effluent irrigation system is based on using a storage pond and solves the problem of the shortcomings of direct land irrigation (Laubach et al., 2015). Both the two ponds of the two-pond system can be used to store the FDE, the solid effluent in the first pond and the liquid fraction in the second pond. This system improves the disadvantages of direct irrigation subject to local climate, soil and farm conditions. The capacity to store FDE allows FDE to be irrigated when the conditions are suitable. For example, effluent collected in the early winter/spring period can be stored when the soil

water content is high during this period (Houlbrooke et al., 2004), to prevent the volume of irrigation exceeding the soil water holding capacity (Horne, 2005).

Deferred irrigation also reduces the risk of nutrient losses into water. Houlbrooke et al., (2004) summarized the average nutrient loss after the deferred irrigation over three lactation seasons. The quantities of N and P loss were 1.1 kg ha^{-1} and 0.2 kg ha^{-1}, accounting for 0.7% of the total N and 0.3% of the total P respectively. Compared with direct irrigation, deferred irrigation minimizes the threat to the aquatic environment and retains the nutrients in FDE. However, this practice still cannot avoid greenhouse gas (GHG) emissions (Laubach et al., 2015).

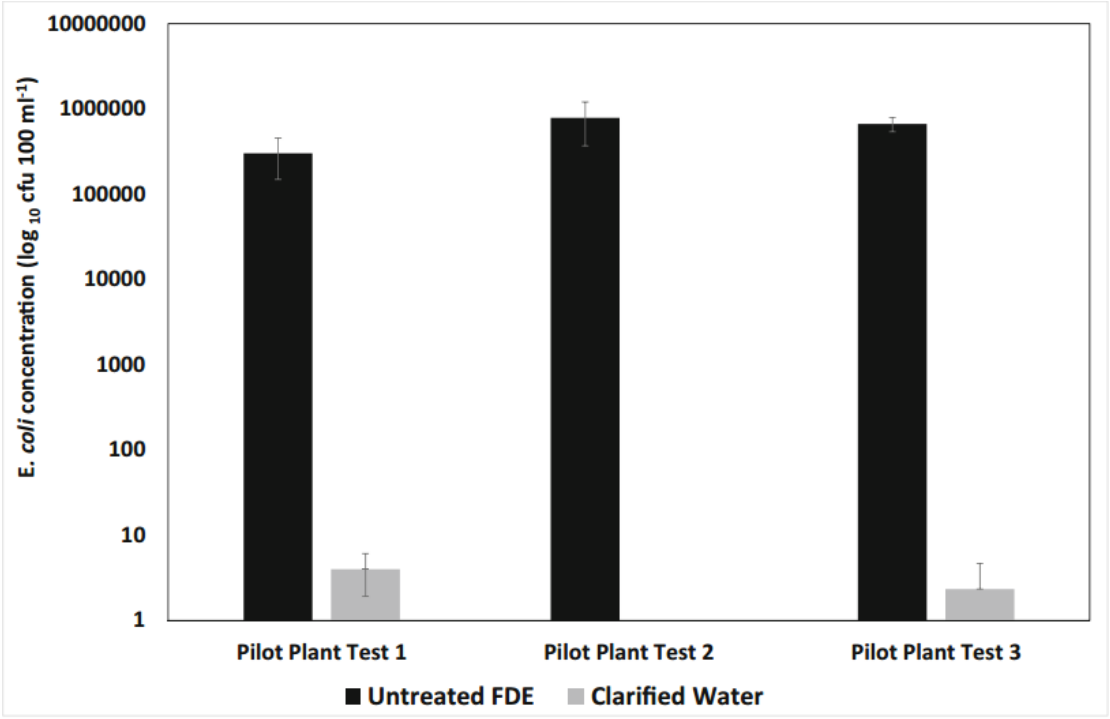
A pre-treatment device has been added to some deferred effluent irrigation systems to prevent solid accumulation. It is composed of a mechanical solid separator or the weeping wall (Laubach et al., 2015). In New Zealand, screw-press and static-screen run-down separators are used as solids separators. They can remove 20-40% of total solids which can be applied to land after drying. The weeping-wall system is increasingly popular in New Zealand, especially in the Southland. It can remove nearly 50% of the total solids.

2.4.4 ClearTech®

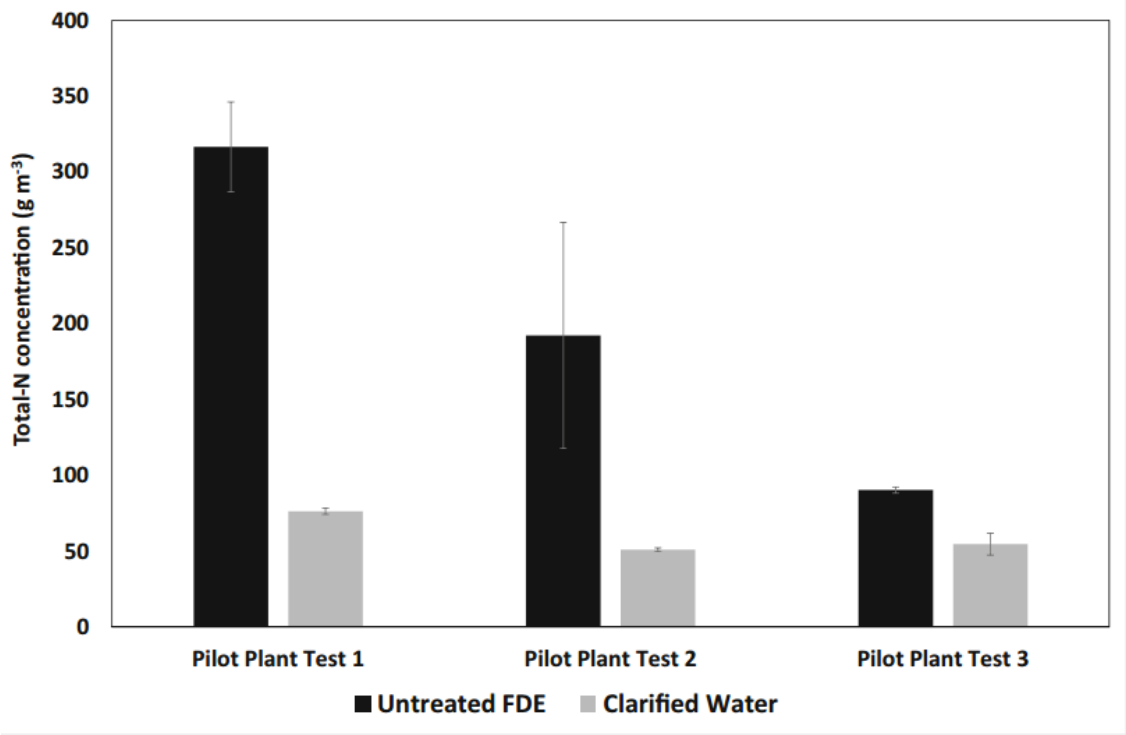
ClearTech® is a new method of treating FDE by separating the water from solids in FDE. The water can be recycled to wash the farmyard and this decreases the volume of FDE needing to be applied to the land (Cameron & Di, 2018; Wang et al., 2018). This new technology uses a coagulant to make the colloidal particles of FDE coagulate and flocculate into flocs (Figure 2.4). These settle out of the liquid fraction due to the gravity. The coagulant can neutralize the negative charges on the solid surface, including soil, dung and organic matter, which prevent them from flocculating. In addition, the mechanism, ‘sweep floc’, produced by adding coagulant into the effluent can also cause the colloids to stick together forming flocs (Cameron & Di, 2018). Thus, FDE may divide into ‘clarified water’ and ‘treated effluent’.

Polyferric sulphate (PFS) is used as the coagulant in this practice as it is effective to treat FDE without the assistance of another hydroxide solutions or additional flocculants (Cameron & Di, 2018). Drinking water treated by PFS is safe for human consumption (Hendrich et al., 2001; Cameron & Di, 2018) . The US Food and Drug Administration (FDA) also approved ferric sulphate as a food additive to improve the iron content of the food.

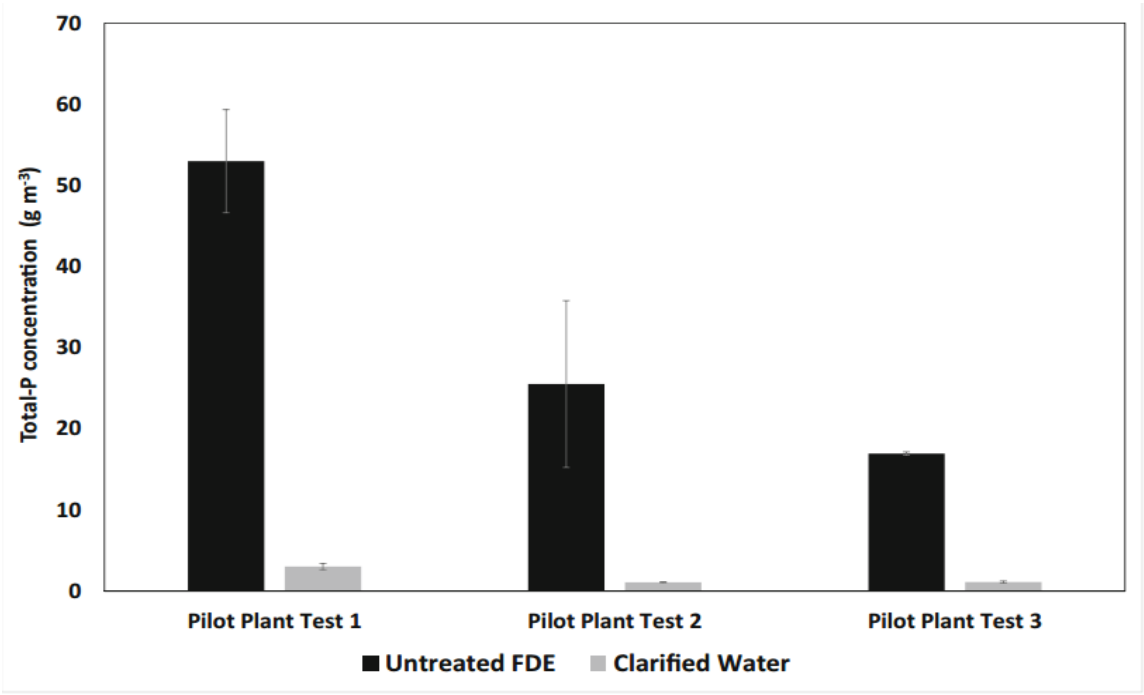
a. *E.coli* concentration



b. Total-N concentration



c. Total-P concentration



d. Dissolved reactive P concentration

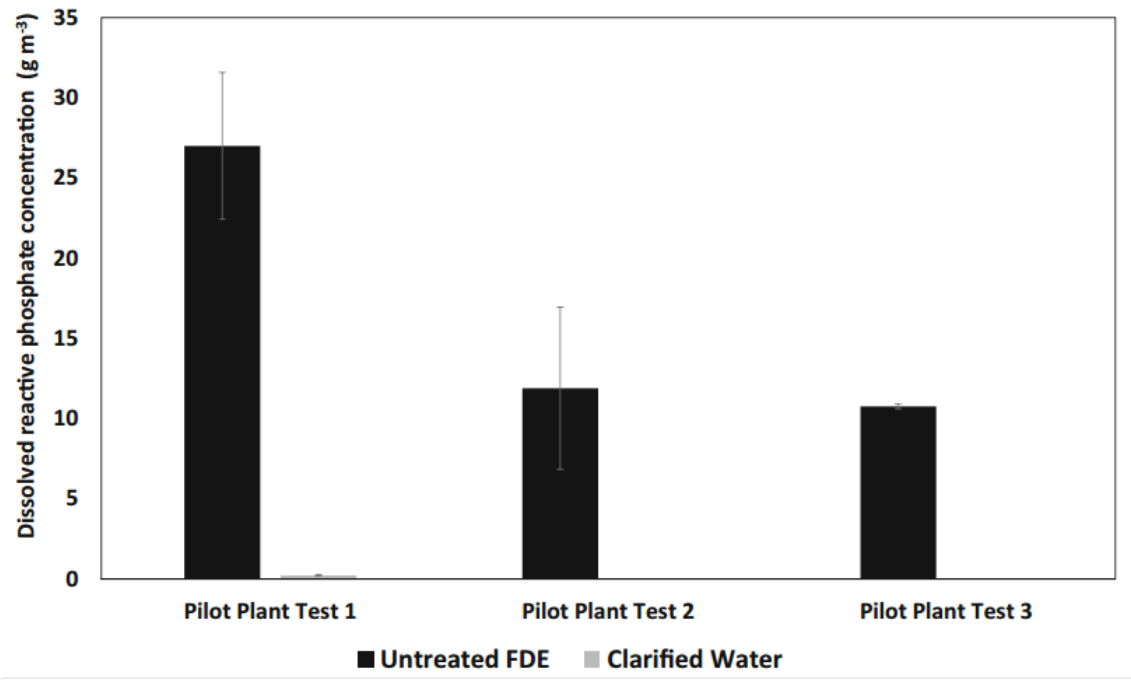


Figure 2.4 Effects of polyferric sulphate (PFS) treatment on farm dairy effluent (FDE) (Cameron & Di, 2018); a) on the E.coli concentration, b) On the total-N concentration, c) on the total-P concentration, and d) on the dissolved reactive P concentration

Cameron and Di (2018) summarized the results of treating 75 different FDE samples collected from 6 farms for 18 months. The results show that with the ClearTech® treatment, the turbidity of FDE decreased significantly from 2096 to 6.3 NTU on average; pH of the FDE also reduced from 7.53 for the untreated FDE, to 5.45 for the clarified water.

Wang et al. (2018) compared the amount of *E.coli*, P and N loss among four different treatments including FDE, treated effluent, a mixture of treated clarified water, and treated effluent and control water (Table 2.7). Results showed that the amounts of *E.coli*, total-P, dissolved reactive phosphate (DRP) and NH_4^+-N loss from treated effluent were less than FDE. However, GHG emissions after the FDE application did not show any significant difference between untreated FDE and treated effluent (Table 2.8) (Wang et al., 2018). Furthermore, the existing studies did not show the effects of the FDE application on the concentrations of macronutrients taken up by the pasture (Cameron & Di, 2018).

Table 2.7 E.coli, P, and N leaching losses over the experimental period (p<0.05) (Wang et al., 2018).

	E.coli (cfu/ha)	P loss (kg p/ha)		N loss (kg N/ha)		
		Total-P	DRP	NO_3^--N	NH_4^+-N	Total-N
FDE	4.21E+10	1.75	0.034	2.14	0.99	3.13
TE	1.31E+10	0.26	0.009	5.92	0.22	6.14
M	9.69E+08	0.18	0.004	7.31	0.28	7.59
Control	7.05E+08	0.28	0.009	2.67	0.16	2.83

Table 2.8 GHG emissions from lysimeters affected by the application of different types of effluents (p<0.05) (Wang et al., 2018).

	N_2O emissions (Kg N_2O-N/ha)	CO_2 emissions (kg CO_2-C/ha)	CH_4 emissions (kg CH_4-C/ha)
FDE	0.44	12817	-0.57
TE	0.61	13046	-0.22
M	0.44	14025	-0.29
Control	0.18	12223	-0.16

2.5 Conclusions

FDE is produced during washing the milking shed and yards. It contains several essential nutrients for plant growth and a large volume of water. Inappropriate management of FDE may lead to serious environmental impacts such as *E.coli*, N and P leaching into the water and GHG emissions. However, a new method of FDE treatment can recycle the liquid fraction to reuse in yard washing, thus decreasing the amount of effluent that need to be stored in pond or irrigated into land. Since the 1970s, direct irrigation of FDE and deferred effluent irrigation have been used instead of direct

discharge into rivers or lakes, however, these systems do not completely solve the environmental problems caused by FDE. The new treatment technology, ClearTech® can decrease the risks of FDE application on the contaminate of water, but its effect on plant growth and soil quality needs further research.

Chapter 3

Materials and Methods

3.1 Introduction

In order to determine the impacts of applying the different effluents on soil fertility, microbial population growth, plant growth, and plant chemical composition, a field experiment was conducted to determine: (1) the effect of applying different effluent treatments on key soil properties and the abundance of ammonia oxidising bacteria (AOB) and archaea (AOA), denitrifying functional genes (*nir*S, *nir*K, and *nos*Z), general agrobacteria and fungi; and (2) the effect of different effluent treatments on plant yield, macronutrient and trace element concentrations in pasture including ryegrass and white clover.

3.2 Experiment Preparation and Setup

3.2.1 Soil and Pasture

The trial field was located at the Lincoln University Research Dairy Farm, which is about 15km southwest of Christchurch (43°38'S, 172°27'E). The soil type used in this study was a Templeton silt loam soil classified as Udic Haplustept (USDA, 2014); Immature Pallic soil (Hewitt, 2010). The pasture contained perennial ryegrass (*Lolium pernne* L.) and white clover (*Trifolium repens* L.).

3.2.2 Farm Dairy Effluent

The farm dairy effluent used for this study was collected from the Lincoln University Demonstration Dairy Farm. ClearTech®, a new farm dairy effluent technology developed at Lincoln University, was used to treat the effluent to separate it into 'treated effluent' and 'clarified water' which can be recycled to wash the farmyard. This technology uses polyferric sulphate (PFS) as a coagulant to neutralise the negative electrical charges on the colloid surfaces so that the colloid particles will be coagulated and flocculated into flocs (Cameron & Di, 2018). Due to gravity, the flocs settle to the bottom to be separated from the liquid. It takes about 3-4 hours from adding PFS into the farm dairy effluent to depositing the flocculated material to the bottom of the treatment tank. The turbidity of the clarified water is less than 50 NTU. The original properties of the untreated effluent, treated effluent and clarified water were analysed for total solids, total N, total NH_4^+-N, total P, dissolved reactive P (DPR), *E.coli*, biochemical oxygen demand (BOD), pH and turbidity (Table 3.1). The untreated effluent, treated effluent and, clarified water were irrigated onto pasture plots separately.

Table 3.1 Original properties of the three different types of effluent used in this study (application 1 and application 2).

Application 1

Chemical property	Untreated FDE	Clarified Water	Treated FDE
Total solid (g m^{-3})	4033.33	2233.33	9133.33
Total nitrogen (g m^{-3})	256.67	137.00	363.33
Ammonium-N (g m^{-3})	107.67	123.67	122.00
Total phosphorus (g m^{-3})	43.00	1.40	81.67
Dissolved reactive phosphorus (g m^{-3})	15.80	0.015	0.02
E.coli (cfu/100ml)	203333	453	433333
BOD (g m^{-3})	1271.11	596.67	1760.00
pH	7.08	6.11	6.34
Turbidity (NTU)	3528.00	77.63	14313.33

Application 2

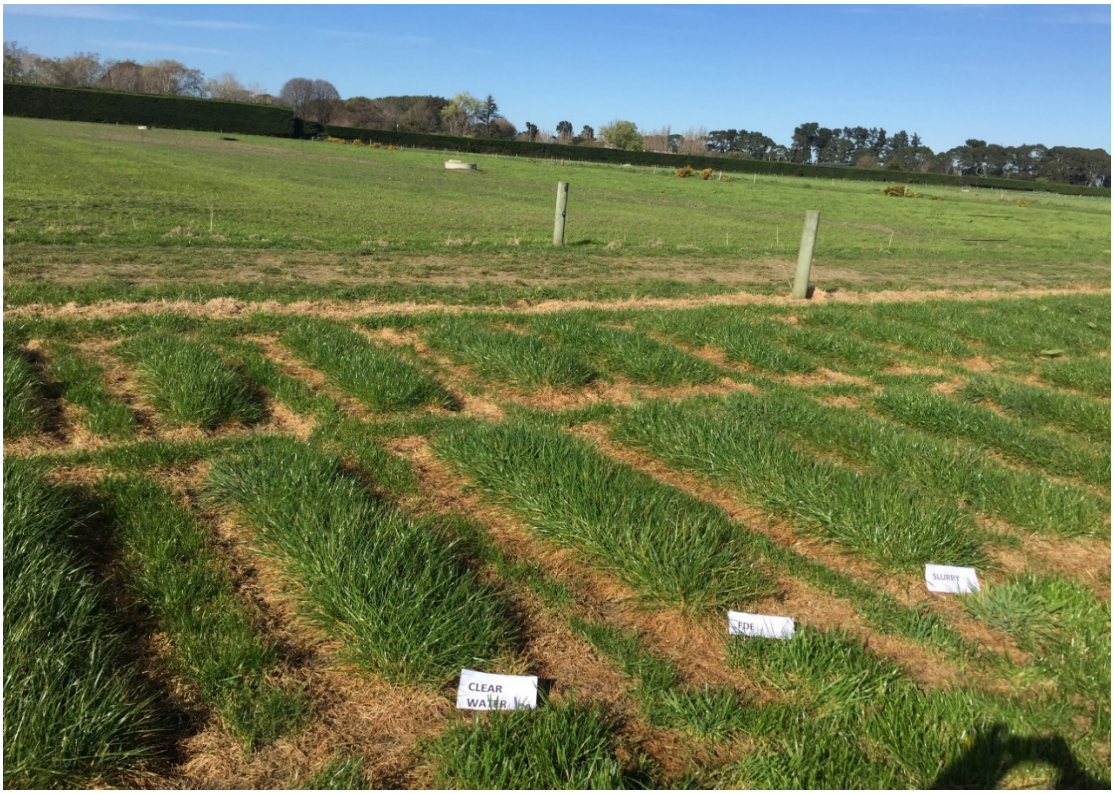
Chemical property	Untreated FDE	Clarified Water	Treated FDE
Total solid (g m^{-3})	5233.33	66.00	13600.00
Total nitrogen (g m^{-3})	326.67	152.00	470.00
Ammonium-N (g m^{-3})	125.67	139.67	131.33
Total phosphorus (g m^{-3})	67.00	0.87	104.00
Dissolved reactive phosphorus (g m^{-3})	13.33	0.20	0.00
E.coli (cfu/100ml)	1600000	36667	1600000
BOD (g m^{-3})	1183.33	490.00	583.33
pH	7.45	5.98	6.33
Turbidity (NTU)	3726.67	27.67	10378.33

3.2.3 Treatments

Four treatments were applied to the plots (Table 3.2). The treatments were: (i) water (control), (ii) untreated effluent, (iii) clarified water, and (iv) treated effluent. The experimental design for this trial was a complete randomized block design. Each treatment had 17 replicate plots which were 0.5 m wide and 2.0 m long with a 0.5 m wide buffer between any two plots (Figure 3.1).

Treatments were applied by hand using watering cans by hands to ensure the effluent could not flow out of the plot area. Treatments were applied on 3 October 2018 and 21 March 2019 and 50 L treatment was applied to each plot per time. The total amount of irrigation over the grazing season was c. 260mm and the total rainfall was 593mm.

a



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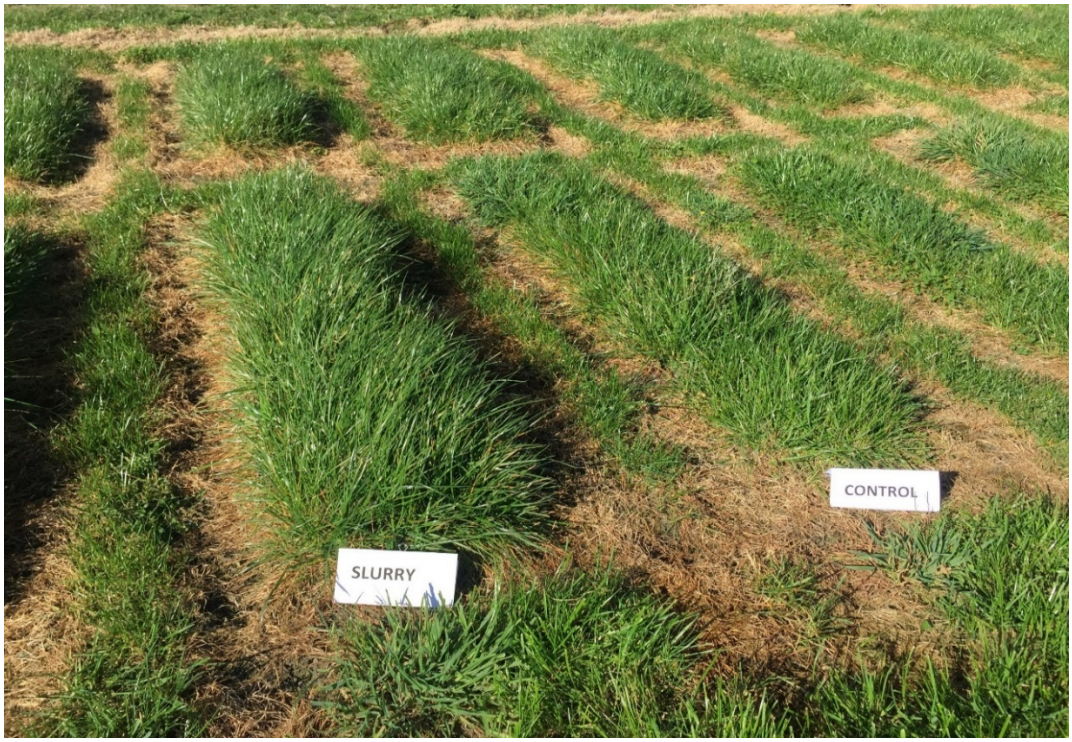

c

Figure 3.1 The randomized blocks for 4 types of treatment (control, clarified water, slurry, and FDE), with 17 replicate blocks of each treatment (a & b, the randomized blocks with different type of treatment; c, 17replicate blocks for 4 treatments). The brown lines use spray method at the edge of each plot.

Table 3.2 Description of the treatments (including application 1 and application 2).

Treatment number	Effluent type	Application 1 N rate (kg N ha^{-1})	Application 2 N rate (kg N ha^{-1})	Replicates
1	Water (control)	0	0	17
2	Untreated Effluent (FDE)	200	200	17
3	Treated Effluent (slurry)	200	200	17
4	Clarified Water	200	200	17

3.3 Soil Sampling and Analysis

Following each treatment application, soil samples were taken after 1 and 14 days, and 1, 2, and three months. Soil samples were taken from six randomly selected plots of each treatment (Plot No. 1, 5, 7, 10, 14, 16) (Figure 3.2). For each plot, the soil was taken by collceting 5 random soil cores (0-7.5 cm depth). Soil samples from the top 7.5 cm were collected, thoroughly mixed, with the roots and stones removed, and sieved through a 5mm sieve (Figure 3.3), for analysis of the concentration of mineral N (including NH_4^+-N and NO_3^--N), soil moisture, pH, organic matter, total C, total N, Olsen-P, extractable S, exchangeable K, Ca, Mg and Na and the abundance of AOB, AOA, denitrifying functional genes (including *nir*S, *nir*K, and *nos*Z), agricultural bacteria, and fungi.

Figure 3.2 Soil samples from 24 plots.

Figure 3.3Figure 3.3 Soil samples collected from the top 7.5 cm, thoroughly mixed, with the roots and stones removed, and sieved through a 5mm sieve.

3.3.1 Soil Moisture

Subsamples of about 20 g were taken from each soil sample and weighed to maintain the soil moisture content during the experiment (Figure 3.4). Subsamples were dried in an oven at 105℃ for 24 hours and then reweighed. The formula for calculating soil moisture content is as follows (Equation 3.3.1.1):

$$\text{Soil moisture content}(\%) = \frac{moist\ weight\ (g) - dry\ weright\ (g)}{dry\ weight(g)} \times 100 \quad (3.3.3.1)$$

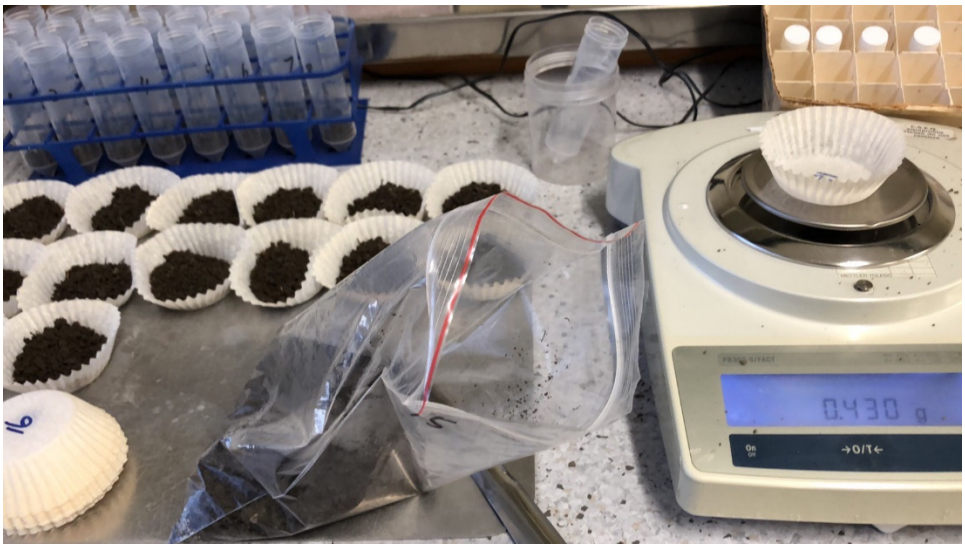

Figure 3.4 Taking soil samples for soil moisture, soil mineral nitrogen and DNA extraction.

3.3.2 Soil Mineral Nitrogen

Subsamples of 5 g of soil weighed from every plot were taken and placed into 50 mL PP Labserv disposable centrifuge tubes (Figure 3.4). 25 mL 2M KCl was added into each tube. The samples were shaken for 60 minutes on a Ratek Platform Mixer, and then centrifuged on Thermo Multifuge 3s-R Centrifuge at 4000 rpm for 10 minutes. Samples were filtered through 110 mm Advantec 5C filter paper into 30 mL PP Labsev white cap vials with two blanks. The extracts were stored in a fridge at -20℃ before being analysed for NH_4^+-N and NO_3^--N using a Flow Injection Analyzer (FIA) (FOSS FIA star 5000 triple channel analyser).

NH_4^+-N was determined by ammonia gas (NH_3) diffusion through the membrane on the FIA. Sodium hydroxide (NaOH) was used to regulate the pH of the sample stream so that ammonium (NH_4^+) and hydroxide ions combined to form NH_3. NH_3 then diffused through the membrane into an indicator stream which changed colour from red to blue when the measurement reached at 590 nm. The change of colour was related to the concentration of NH_4^+ in the sample.

To determine the concentration of NO_3^--N, NO_3^- was reduced to NO_2^- by a cadmium (Cd)-filled coil in the FIA. Then an azo dye compound was formed by the reaction of NO_3^- with sulphanilamide/NED. The compound intensity was measured by spectrophotometry at 540 nm.

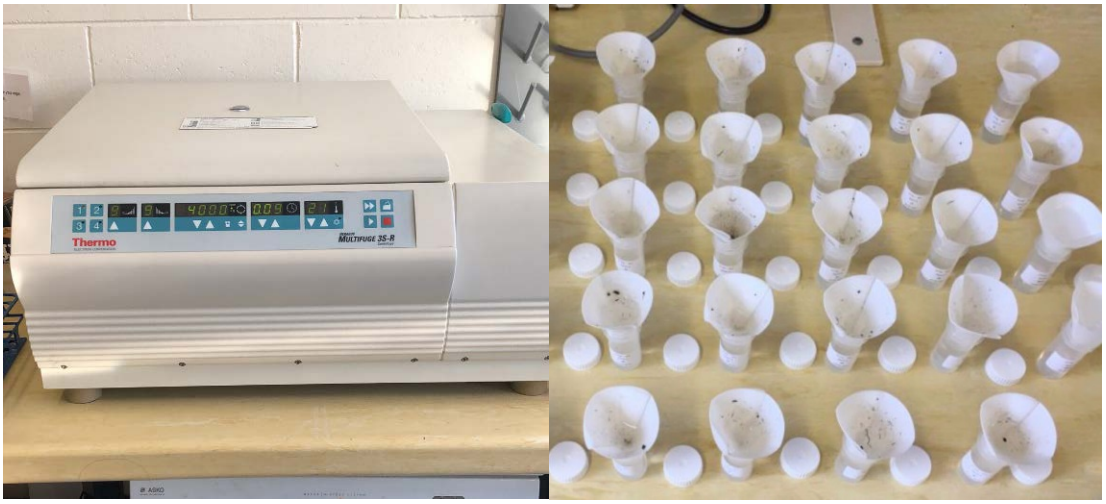

Figure 3.5 KCl extraction. Left, soil samples centrifuged; right, filtering.

3.3.3 Functional Gene Abundance Qualification

DNA extraction

DNA was extracted by using the NucleoSpin® Soil Kit (Macherey-Nagel, Duren, Germany). For each plot, 0.25g soil sample was taken and placed in a NucleoSpin® bead tube. Soil samples with 700 µL buffer SL2 and 150 µL enhancer were processed in the FastPrep bead for 1 min to homogenise the samples. After homogenising well, samples were centrifuged at the speed of 11000g for 2 min. The supernatant was transferred to a new tube and 150 µL Buffer SL3 was added. The sample was vortexed for 5 sec to mix the supernatant and buffer evenly and incubate at 4℃ for 5 min before being centrifuged at a speed of 11000g for 1 min. After centrifugation, 700 µL of supernatant was loaded up from the previous tube onto a NucleoSpin® Inhibitor Removal Column (red ring) in a Collection Tube and centrifuged for 1 min at 11000g again. If the supernatant in the tube was more than 700 µL, the previous step needed to be repeated. The supernatant was leached from the inhibitor removal column and 250 µL Buffer SB was added and then vortexed for 5 sec. Then, 550 mL of the mixture was loaded on to a new NulceoSpin® Soil Column (green ring) in a Collection Tube and centrifuged for 1 min at 11000g. The flowthrough was discarded after centrifugation. This step was repeated with the remaining sample. After discarding the flowthrough, 500 µL Buffer SB was added into the NuclesSpin® Soil Column. The flowthrough was discarded after centrifuging 30 sec at 11000g. 550 µL Buffer SW1 added into the NuclesSpin® Soil Column. The flowthrough was discarded after centrifuging 30 sec at 11000g. Then 700 µL Buffer SW2 was added into the NucleoSpin® Soil Column and vortexed for 2 sec. The flowthrough was discarded after centrifuging 30 sec at 11000g. This step was repeated once. The sample was centrifuged for 2 min at 11000g to ensure there was no liquid left in the NucleoSpin® Soil Column. Then, the NucleoSpin Soil Column was placed into a new Collection Tube to elute the DNA in the sample by adding 100 µL Buffer Se to the column. The lid of the column was left open for 1min at room temperature for incubating. After incubation, the sample was centrifuged for 30 sec at 11000g, and the eluted DNA was used in the downstream applications. The purified DNA was stored at -20℃ before being analysed by real-time PCR.

Real-time qPCR Analysis

A Rotor-Gene™ 6000 (Corbett Life Science) was used for real-time PCR to measure the abundance of AOB, AOA, nirS, nirK, nosZ (I), nosZ (II), general agrobacteria and fungi. All PCR reactions used CAS-1200 Robotic liquid handling system (Corbett Life Science, Australia). The DNA extraction samples of soil were diluted by adding deionized water to achieve a ration of 1:10. Table 3.3 shows the primer pairs, final concentrations of each primer pair combination, and temperature profile used in the qPCR analysis. 8 µL of SYBR Premix Ex Taq (TaKaRa, Nori Biotech, Auckland, New Zealand) was mixed with the 1.5 µL aliquot of each 1:10 diluted template soil genomic DNA. Then 16 µL of reaction

mixture was added to 0.4-1.6 µL for each primer (as described in Table 3.3). The data was analysed by Roter Gene 6000 series software 1.7. A melting curve analysis was used to identify the specificity of PCR product by measuring the fluorescence continuously with the temperature increasing from 72 to 99 ℃.

The previous process was used to amplify the extracted DNA of AOB, AOA, *nir*S, *nir*K, *nos*Z (I), *nos*Z (II), general agrobacteria, and fungi from soil samples. A clean-up kit (Axygen) was used to purify the PCR products which then were cloned into the pGEM-T Easy Vector (Promega, Madison, WI). According to the manufacturer's instruction, the clones were transformed into Escherichia coli JM109 competent cells (Promega) after cloning. E.coli cells with the clones were cultured on LB plates at a temperature of 37℃ overnight. 10-15 bacterial colonies grown on the LB plates were inoculated into a 3 mL LB broth medium, respectively. Then an incubator-shaker was used overnight at 37℃ and set out a speed of 250 rpm. The PureLink™ Quick Plasmid Miniprep Kit (Life Technologies, Auckland, New Zealand) was used to extract the plasmids from the overnight cultures. The plasmids were used as the templates in the reactions of PCR with SP6 and T7 primers, which were used to generate the PCR amplicons of each PCR.

Table 3.3 The primer pairs and PCR conditions used in the real-time qPCR analysis.

Target group	Primer name	Sequence (5′-3′)	Length of implication (bp)	Primer final concentration (nM)	Thermal profile	Amplification efficiency (R^2>0.99) (%)	References
Bacterial *amo*A	*amo*A1F	5′-GGGGTTTCTACTGGTGGTGGT-3′	491	250	95 ℃ for 2 min- × 1 cycle;	96-98	(Rotthauwe et al., 1997)
	*amo*A2R	5′-CCCCTCKGSAAAGCCTTCTTC-3′			95℃ for 20 s, 57℃ for 30 s, 72℃ for 30 s, 85℃ for 10 s- × 40 cycles;		
Archaeal *amo*A	Arch-*amo*AF	5′-STAATGGTCTGGCTTAGACG-3′	635	750	95 ℃ for 2 min- × 1 cycle;	92-94	(Francis et al., 2005)
	Arch-*amo*AR	5′-GCGGCCATCCATCTGTATGT-3′			95℃ for 20 s, 55℃ for 20 s, 72℃ for 30 s, 85℃ for 10 s- × 40 cycles;		
*nir*S	Cd3af	5′-GTSAACGTSAAGGARACSGG-3′	410	750	95 ℃ for 2 min- × 1 cycle;	93-95	(Michotey et al., 2000)
	R3cd	5′-GASTTCGGRTGSGTCTTGA-3′			95℃ for 45 s, 55℃ for 45 s, 72℃ for 45 s, 85℃ for 20 s- × 40 cycles;		(Throbäck et al., 2004)
*nir*K	FlaCu	5′-ATCATGGTSCTGCCGCG-3′	474	780	95 ℃ for 2 min- × 1 cycle;	98-100	(Hallin & Lindgren, 1999)
	R3Cu	5′-GCCTCGATCAGRTTGTGGTT-3′			95℃ for 20 s, 55℃ for 30 s, 72℃ for 30 s, 85℃ for 10 s- × 40 cycles;		
*nos*Z (I)	*nos*Z-F	5′-CGYTGTTCMTCGACAGCCAG-3′	424	750	95 ℃ for 2 min- × 1 cycle;	94-99	(Kloos et al., 2001)
	*nos*Z1622R	5′-CGSACCTTSTTGCCSTYGCG-3′			95℃ for 20 s, 55℃ for 30 s, 72℃ for 30 s, 85℃ for 15 s- × 40 cycles;		(Throbäck et al., 2004)
*nos*Z (II)	*nos*Z-II-F	5′-CTIGGICCIYTKCAYAC-3′	698	1000	95 ℃ for 2 min- × 1 cycle;	76-81	(Jones et al., 2013)
	*nos*Z-II-R	5′-GCIGARCARAAITCBGTRC-3′			95℃ for 20 s, 50℃ for 30 s, 72℃ for 45 s, 85℃ for 10 s- × 40 cycles;		
general agrobacteria	1369F	5′-CGGTGAATACGTTCYCGG-3′	100	0.312	94 ℃ for 2 min- × 1 cycle;	95-101	(Suzuki et al., 2000)
	1492R	5′-GGWTACCTTGTTACGACTT-3′			94℃ for 10 s, 56℃ for 30 s- × 40 cycles;		
Fungi	FR1	5′-AICCATTCAATCGGTAIT-3′	390		95 ℃ for 10 min- × 1 cycle;	67-103	(Prevost-Boure et al., 2011)
	FF390	5′-CGATACGAACGAGACCT-3′			95℃ for 15 s, 50℃ for 30 s, 70℃ for 60 s- × 40 cycles;		

3.4 Plant Sampling

The pasture from all 17 replicate plots of the 4 different effluent treatments were harvested following typical grazing schedules. The weight of the fresh plant from each plot was recorded (Figure 3.6). After harvest, the plant was put in a drying oven to dry, and then ground. The ground pasture samples were used to determine the concentrations of macronutrient and trace elements.

Figure 3.6 Recording the harvest for each plot.

Chapter 4

Effect of treated and untreated farm dairy effluents on soil fertility, microbial population growth, plant growth, and plant chemical composition

4.1 Introduction

Farm dairy effluent (FDE) is a mixture of cow urine and faeces deposited in the milking shed and diluted by wash-down water, detergents, acids, and other cleaners (Chung et al., 2013; Laubach et al., 2015). In general, FDE consists of about 10% cow excreta and 86% wash-water (Gibson, 1995) and also contains a variety of valuable nutrients for plant growth such as nitrogen (N), phosphorus (P), organic carbon (C), potassium (K) and, sulphur (S) (Li et al., 2014;Hawke & Summers, 2006). The compositional variations of FDE are dependent on the cow's age and breed, milking time, fertiliser conditions and feed quality (Cooke et al., 1979; Goold, 1980; Longhurst et al., 2000); Hawke & Summers, 2006.

In New Zealand, there is an increasing amount of FDE generated by the intensification of the dairy industry. However, inadequate management of FDE has resulted in serious environmental issues, including negative effects on the quality of surface water, risks of high nitrate content in groundwater, and the problem of odour (Longhurst et al., 2000; Ali et al., 2006). In addition, the large volumes of water used by agriculture and the low water use efficiency also cause significant environmental and resource problems (Cameron & Di, 2018). Thus, several FDE management systems, such as two-pond systems, direct land irrigation, and deferred effluent irrigation, have been developed to recycle FDE for using irrigation. Irrigation of FDE has been widely used in the New Zealand dairy industry to protect surface water quality from direct contamination by discharging into the water, to improve the soil fertility, and to reduce the waste of freshwater since last century (Cameron et al., 2014; Cameron & Di, 2018).

ClearTech® is a new technology to treat FDE by using a coagulant, polyferric sulphate (PFS), to coagulate the colloidal particles in FDE into flocs which are precipitated by gravity (Cameron & Di, 2018). Thus, the FDE is separated into 'clarified water' and 'treated effluent'. The clarified water is recycled to wash the milking yard while the treated effluent is applied to land to recycle the nutrient. However, the effects of these different types of effluents, e.g. treated effluent and clarified water when applied to soil on soil fertility, microbial population growth, plant growth, and plant chemical

composition are largely unknown. The results from a field experiment to assess the effects of different effluents on soil and pasture growth parameters are reported in this Chapter (4).

4.2 Materials and methods

4.2.1 Experimental methods

The materials and methods have been described in detail in Chapter Three. Only a brief summary is presented here.

A field experiment was conducted to determine: (1) the effect of applying different effluent treatments on key soil properties and the abundance of ammonia oxidising bacteria (AOB) and archaea (AOA), denitrifying functional genes (*nir*S, *nir*K, and *nos*Z), general agrobacteria and fungi; and (2) the effect of different effluent treatments on plant yield, macronutrient and trace element concentrations in pasture including ryegrass and white clover.

4.2.2 Experimental design

Four types of treatments, including: (i) water (control); (ii) untreated effluent (UE); (iii) clarified water (CW); and (iv) treated effluent (TE), were applied to field plots. Each treatment had 17 replicates, and the treatments were arranged in a randomised block design.

4.2.3 Statistical analysis

All variables of the randomised complete block design were statistically analysed by analysis of variance. Besides, for microbial functional genes, logarithmic transformation of data values was carried out before analysis to ensure the homogeneity of variance assumption was satisfied.

4.3 Results

4.3.1 Effects on soil fertility

Organic Matter

The application of the TE resulted in significantly higher soil organic matter contents compared with the application of UE, CW or in the control ($P<0.05$). There was no significant difference in the organic matter contents among the control, UE and CW treatments (Fig. 4.1).

Total C

The overall trend of total C is similar to that of organic matter (Fig. 4.2). That is the total C content was significantly higher in the TE treatment than in the other treatments ($P<0.05$), and there were no differences in total C content among the UE, CW and control treatments.

Total N

Similar to the organic matter content, the total N content was also significantly higher in the TE treatment than in the other treatments ($P<0.05$), and there was no difference among the other treatments (Fig. 4.3).

C: N ratio

There was no significant difference in C: N ratio between the control and the other three different types of effluent treatments (Fig. 4.4).

Organic S

The application of the TE and the CW increased the soil organic S contents above those in the control and the UE treatment, particularly following the second application (Fig. 4.5). The organic S contents then declined with time after the second application.

Sulphate Sulphur

The application of the TE and CW also significantly increased soil sulphate S contents above those in the control and the UE treatment ($P<0.05$). The sulphate S was also higher in the CW treatment than in the TE treatment after the second application. The sulphate S levels declined sharply with time after the second application (Fig. 4.6).

Soil pH

The application of the effluent treatments kept the soil pH between 5.5 and 6 during the experiment period (Fig. 4.7). After the second application, the soil pH values in the CW and TE were slightly lower than in the control and the UE and then rose back above 5.5 a month later, reaching similar values at the last sampling.

Olsen P

The application of the TE led to significantly higher Olsen-P levels compared with the control, CW and the FDE treatments ($P<0.05$, Fig. 4.8). There was no significant difference in Olsen P in CW, UE and the control ($P>0.05$). The Olsen P then declined gradually with time after the second application.

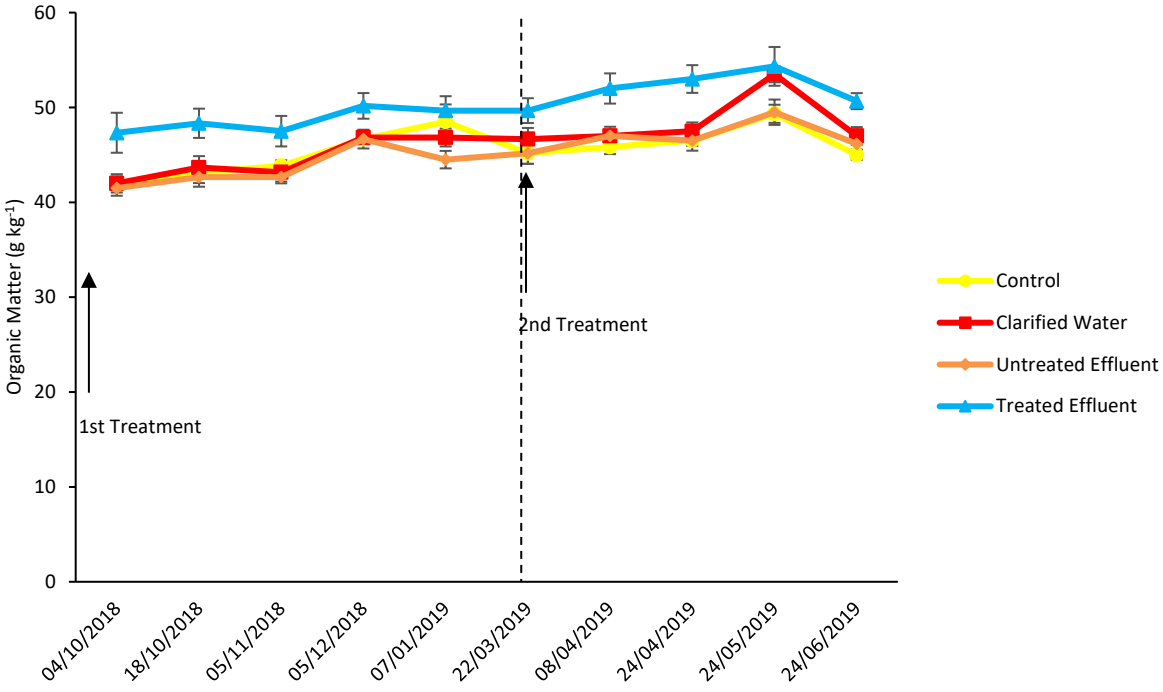

Figure 4.1 The organic matter content of the soil. The error bars represent the standard error of the mean (n=6).

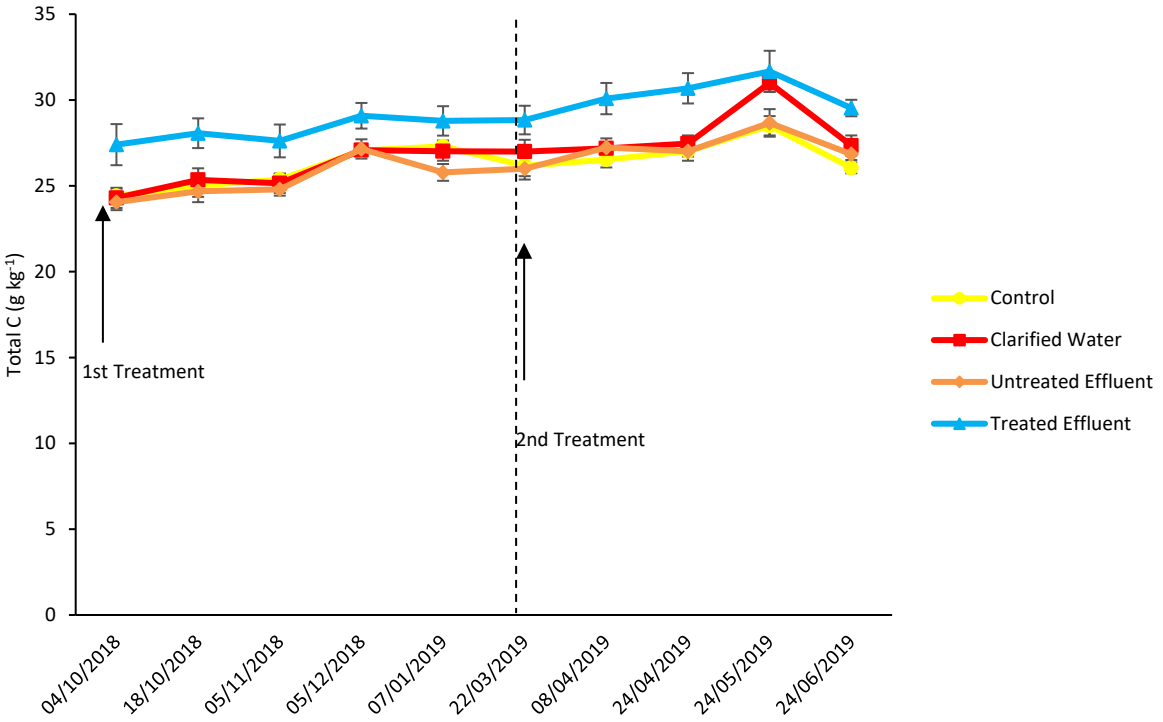

Figure 4.2 The total C content of the soil. The error bars represent the standard error of the mean (n=6).

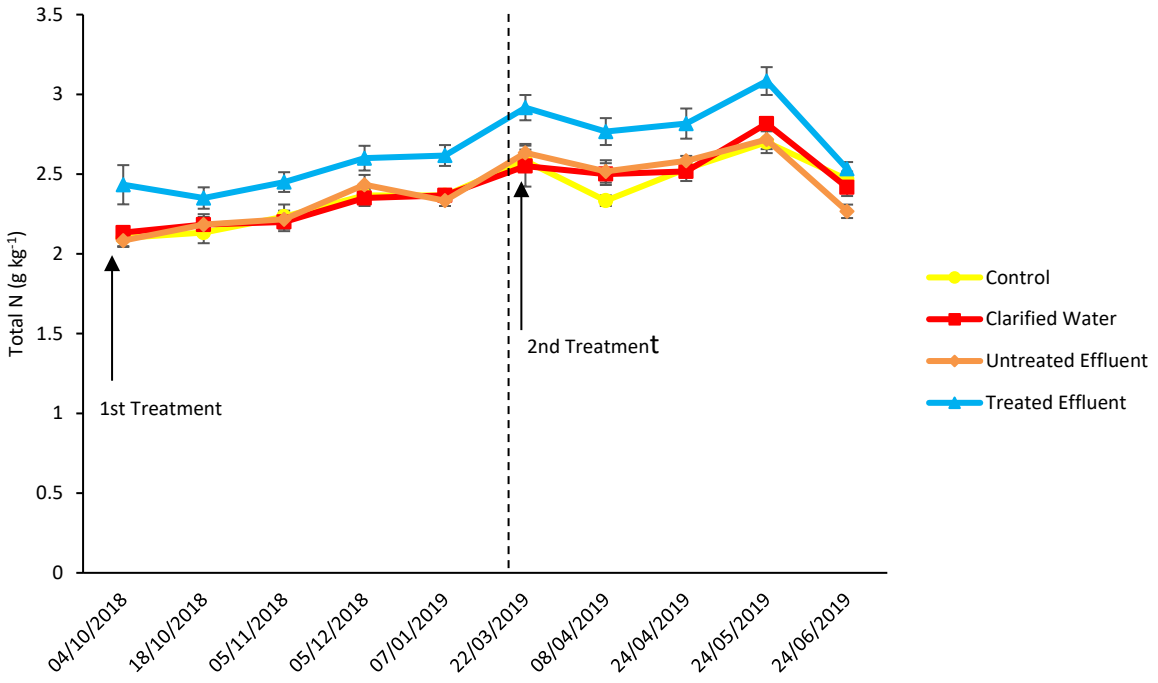

Figure 4.3 The total N content of the soil. The error bars represent the standard error of the mean (n=6).

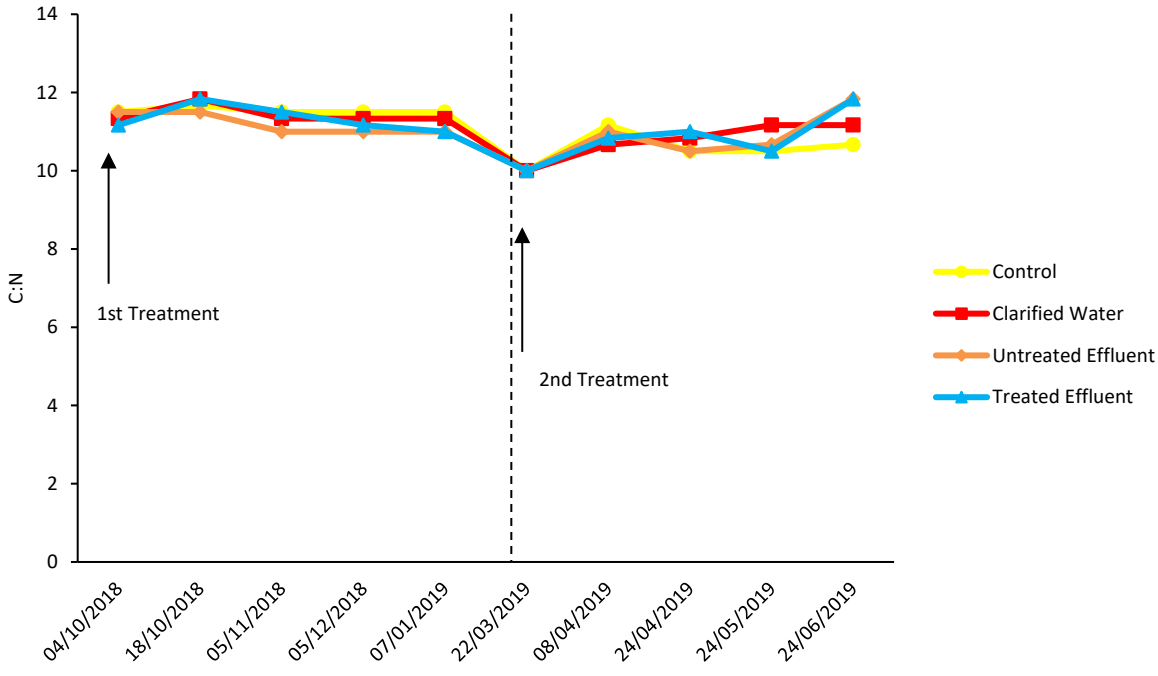

Figure 4.4 C: N ratio. The error bars represent the standard error of the mean (n=6).

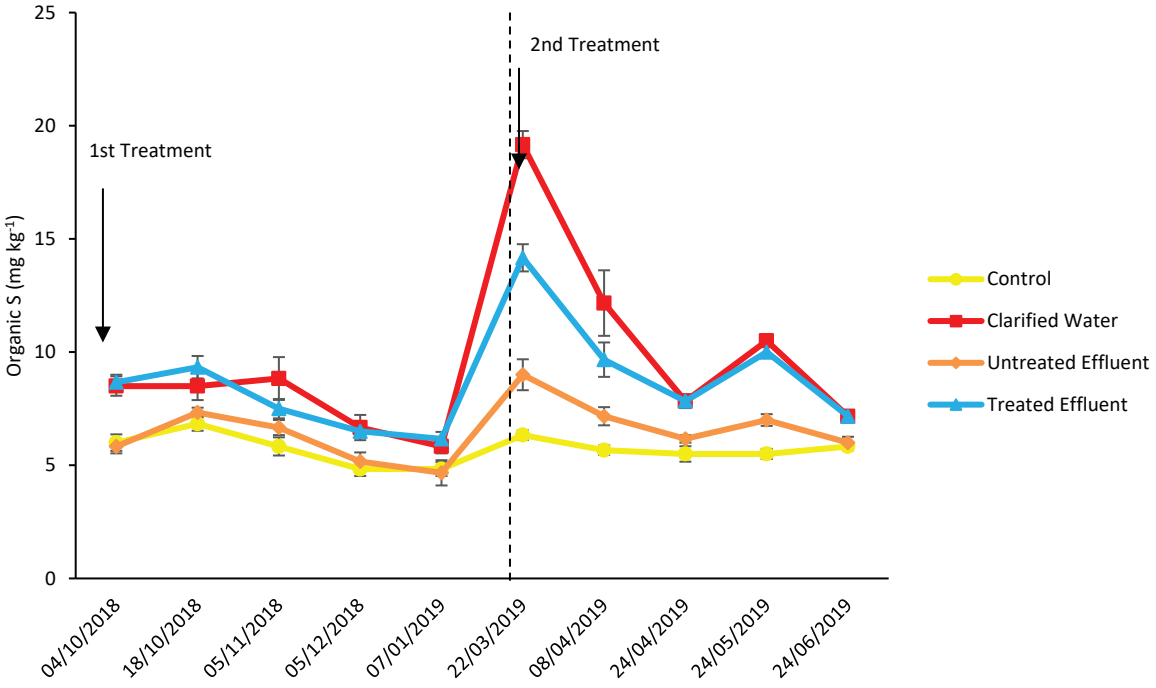

Figure 4.5 The organic S content of the soil. The error bars represent the standard error of the mean (n=6).

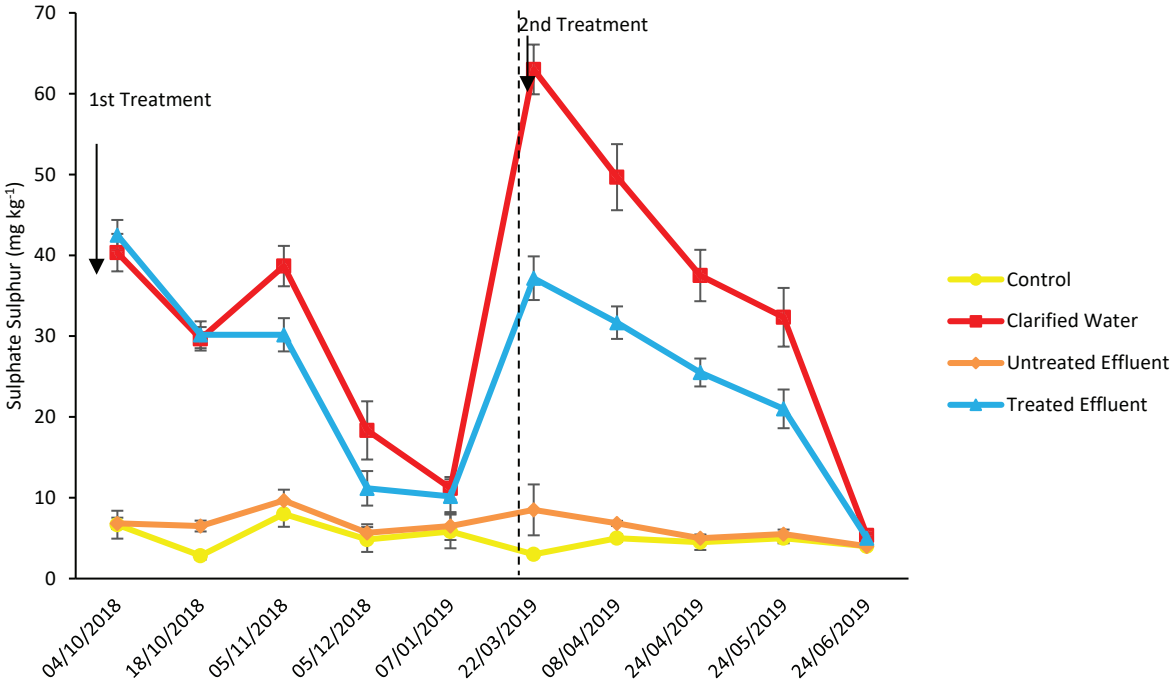

Figure 4.6 The sulphate sulphur content of the soil. The error bars represent the standard error of the mean (n=6).

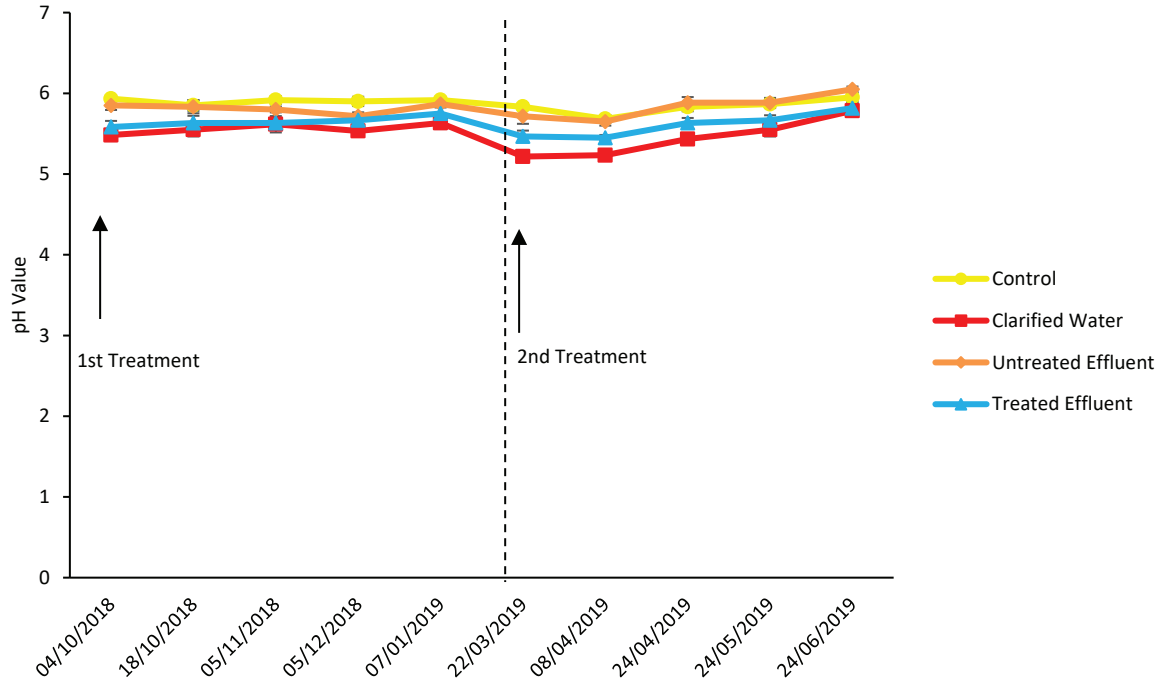

Figure 4.7 The pH value of the soil. The error bars represent the standard error of the mean (n=6).

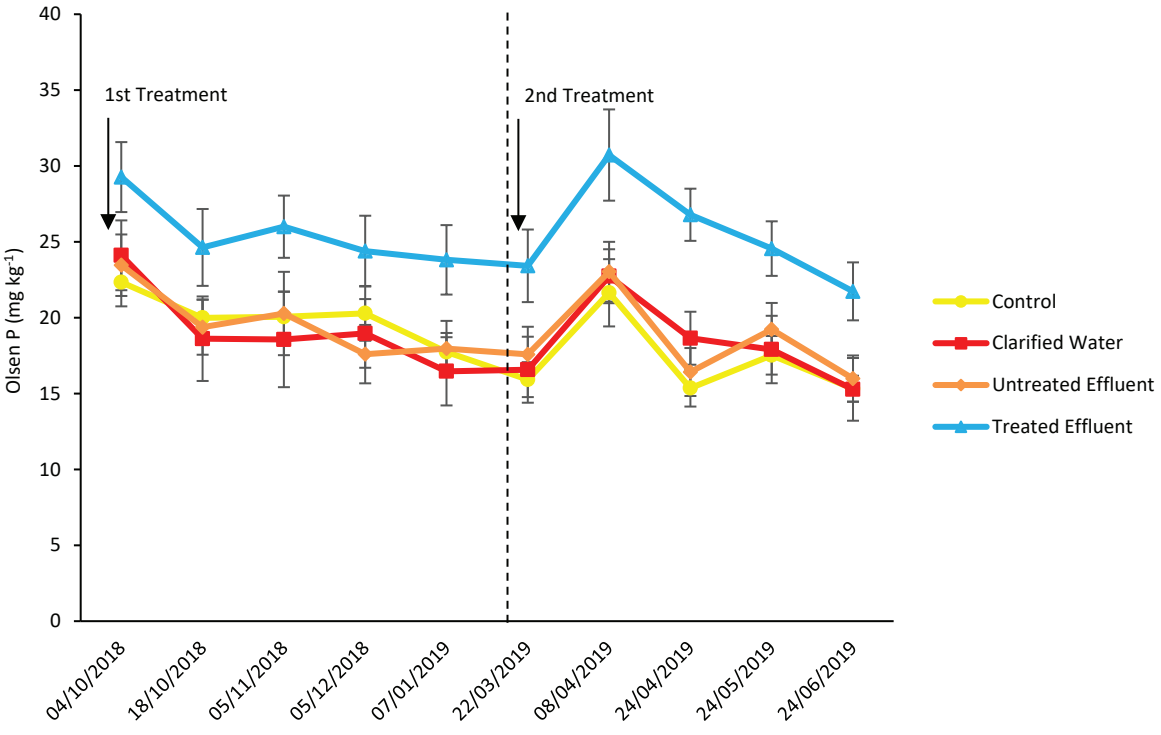

Figure 4.8 The Olsen P content of the soil. The error bars represent the standard error of the mean (n=6).

Ammonium-N

The concentration of NH_4^+-N increased after each application and then dropped sharply in the first 15 days in the effluent treatments. After the second application, the NH_4^+-N concentration from CW was particularly high (8.09 mg kg^{-1} soil) while the NH_4^+-N concentrations in UE and TE were only 1.44 and 2.62 mg kg^{-1} soil.

Nitrate-N

Small increases in NO_3^--N concentration were recorded straight after the application of the three effluents ($P<0.05$). However, the NO_3^--N concentration then declined to similar values in the different treatments (Fig. 4.10).

CEC and exchangeable bases

There was no significant difference in CEC between the different treatments following both applications of the effluents (Fig. 4.11).

Generally speaking, there were no major differences in the soil exchangeable soil Ca^{2+} among the different treatments (Fig. 4.12). Similarly, there were no major differences in the soil exchangeable Mg^{2+} between the different treatments following the application of the effluents (Fig. 4.13). However, the application of all three effluents significantly increased the soil exchangeable K^+ and Na^+ concentrations above those in the control plots (Fig. 4.14 and 4.15).

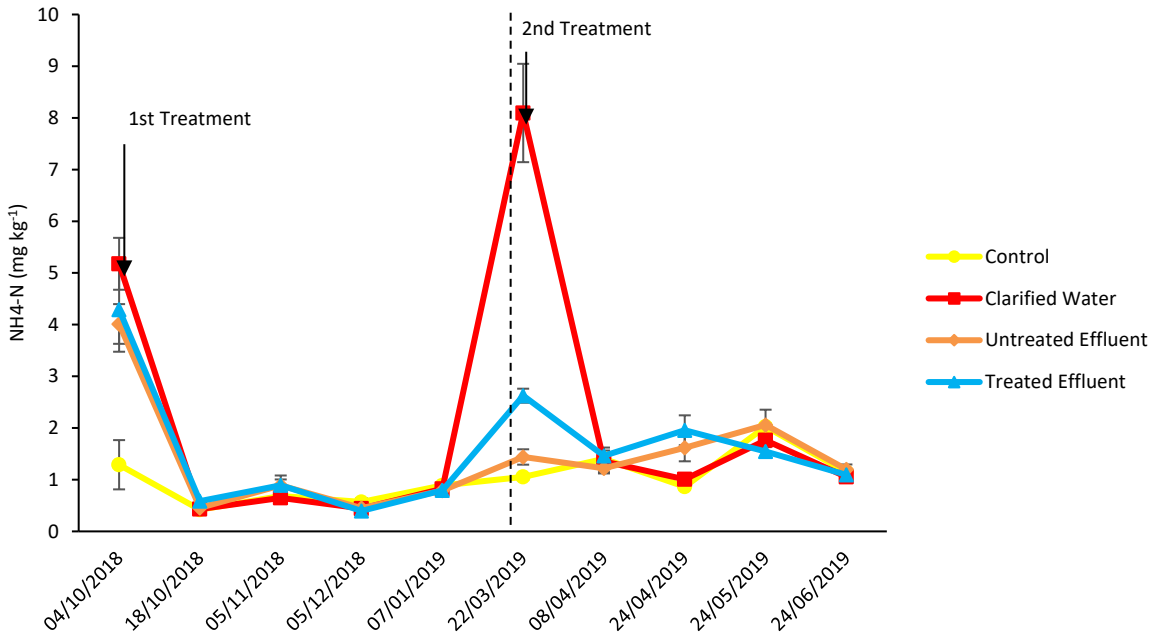

Figure 4.9 The ammonium-N concentration of the soil. The error bars represent the standard error of the mean (n=6).

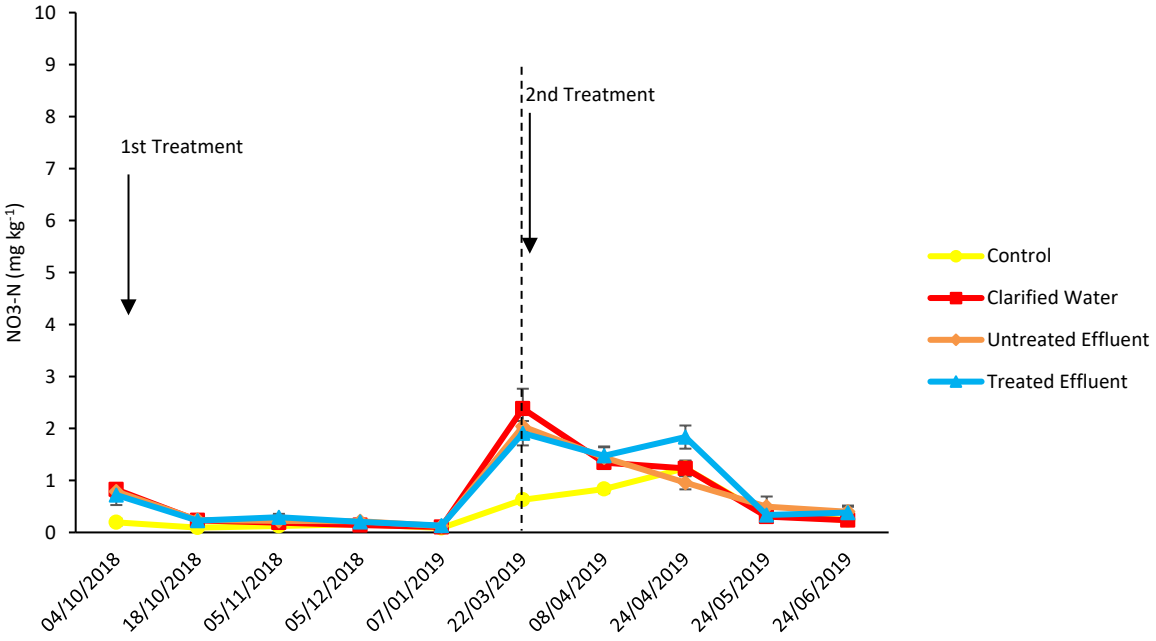

Figure 4.10 The nitrate-N concentration of the soil. The error bars represent the standard error of the mean (n=6).

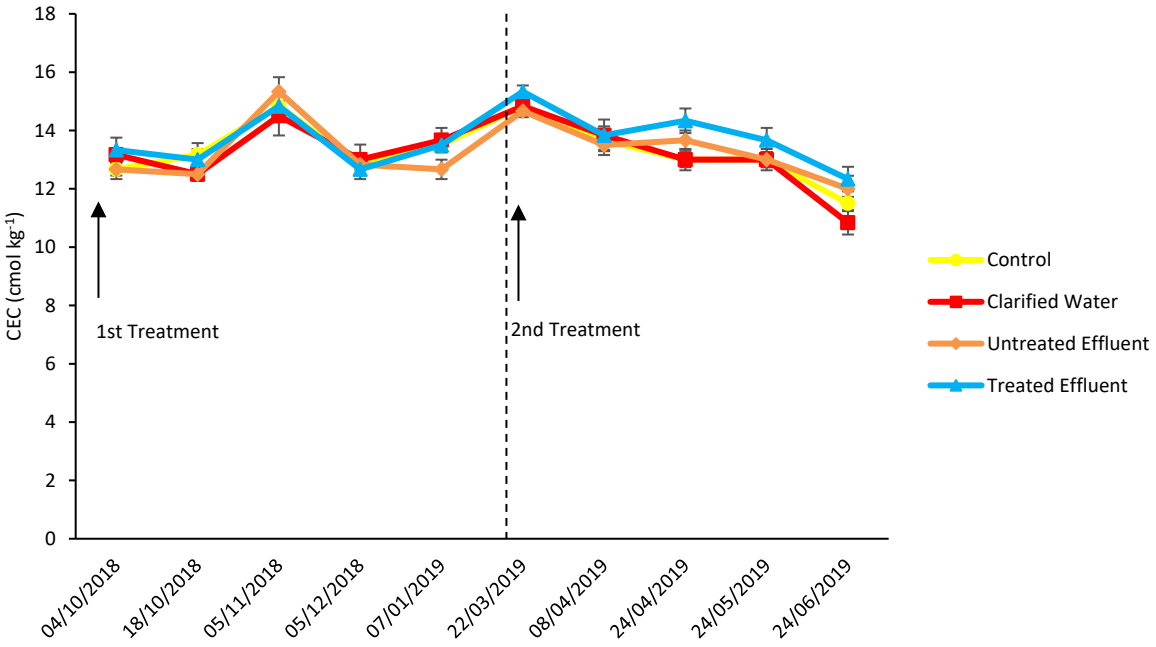

Figure 4.11 CEC in the soil. The error bars represent the standard error of the mean (n=6).

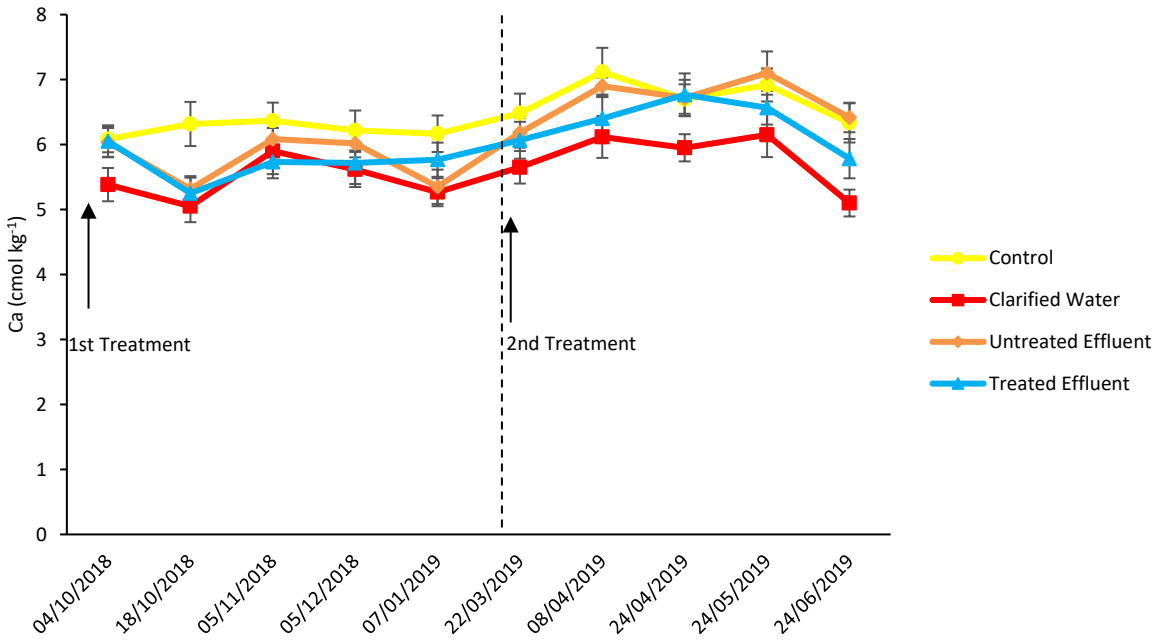

Figure 4.12 Concentration of Ca^{2+} in the soil. The error bars represent the standard error of the mean (n=6).

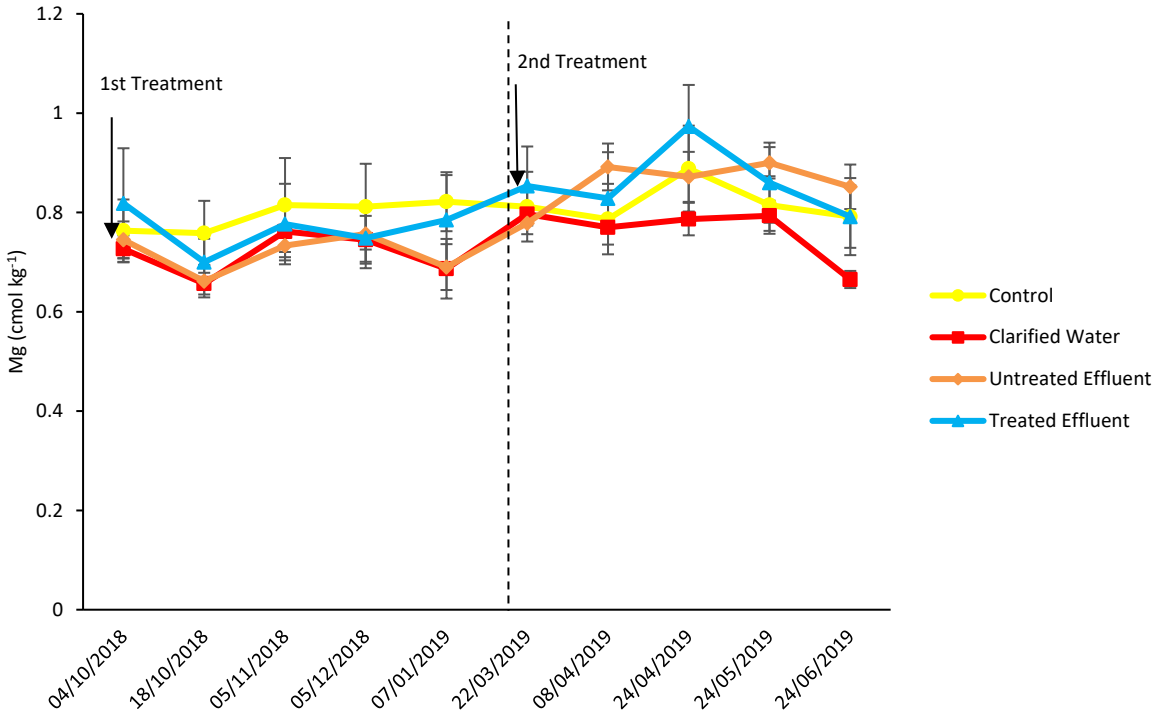

Figure 4.13 Concentration of Mg^{2+} in the soil. The error bars represent the standard error of the mean (n=6).

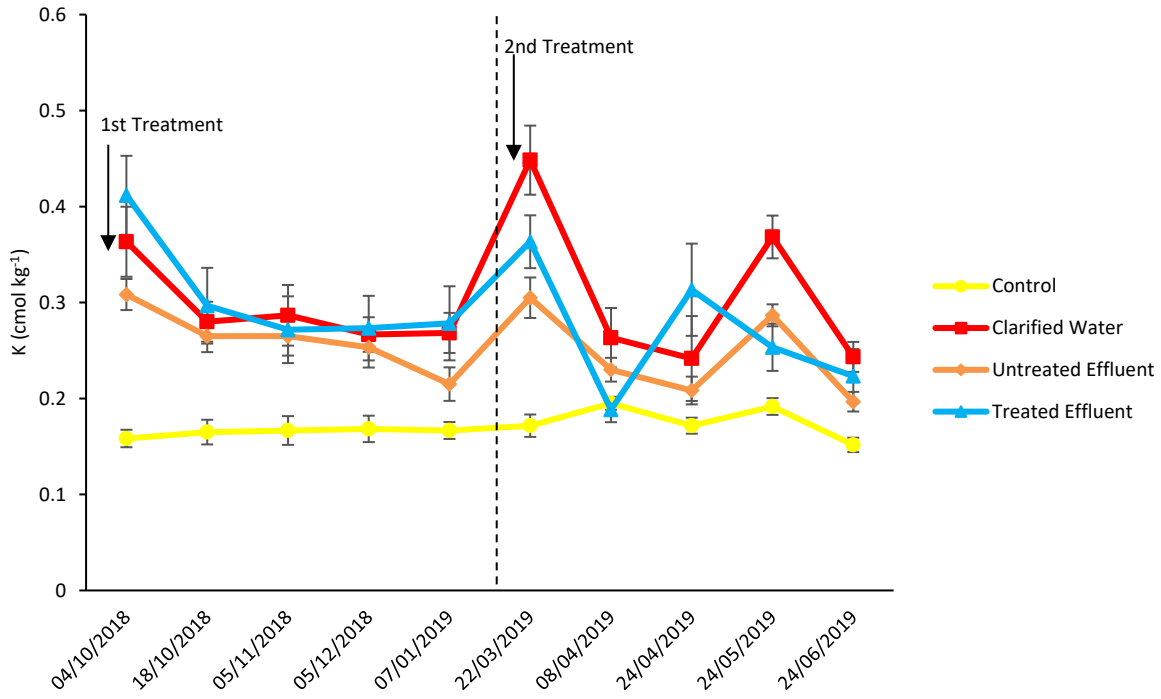

Figure 4.14 Concentration of K^+ in the soil. The error bars represent the standard error of the mean (n=6).

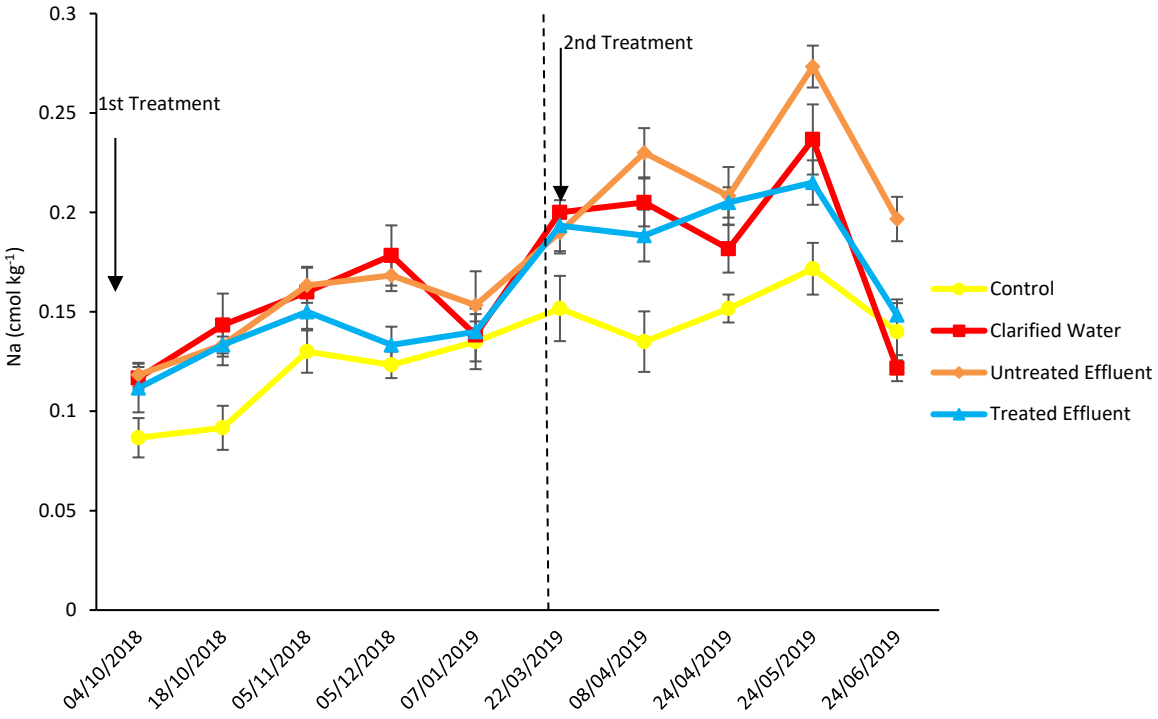

Figure 4.15 Concentration of Na^+ in the soil. The error bars represent the standard error of the mean (n=6).

4.3.2 Functional gene abundance

AOB

The application of the effluent treatments increased the AOB *amo*A gene copy numbers in the first month after the first application and decreased sharply for the next two months (Fig. 4.16). After the second application, the AOB *amo*A gene copy numbers remained steady at a low level in the first month, began to increase in the second month and then decreased again. The AOB *amo*A gene copy numbers from CW, UE and TE treatments were significantly higher than those in the control ($P<0.05$).

AOA

The AOA *amo*A gene copy numbers decreased sharply two months after the first application and increased gradually one month later after the second application (Fig. 4.17). The AOA *amo*A gene copy numbers from CW, TE and UE were significantly higher than those in the control during the entire period of this study ($P<0.05$).

***nir*S**

After the first application of the effluent treatments, the *nir*S gene copy numbers remained steady during the first month and then decreased sharply (Fig. 4.18). In contrast, the *nir*S gene copy numbers kept increasing after the second application. The application of TE resulted in higher copy numbers than in the other treatments after the second application($P<0.05$). There was no significant difference in the *nir*S gene copy numbers between the control, CW and UE treatments ($P>0.05$).

***nir*K**

The changing patterns of *nir*K gene copy numbers after the first application were similar to those of *nir*S gene copy numbers (Fig. 4.19). After the second application, *nir*K gene copy numbers began to increase one month later. There was no significant difference in *nir*K gene copy numbers among control, CW and UE treatments ($p>0.05$); however, the application of TE resulted in higher copy numbers of *nir*K than in the other treatments ($P<0.05$).

***nos*Z I**

After the first application, *nos*Z I gene copy numbers remained steady for the first two months and declined dramatically in the third month (Fig. 4.20). The changing patterns of *nos*Z I gene copy numbers after the second application were similar to those of *nir*S gene copy numbers. There was no significant difference in *nos*Z I gene copy numbers among control, CW and UE treatments ($P>0.05$); however, the application of TE resulted in higher copy numbers of *nos*Z I than in the other treatments ($P<0.05$).

*nos*Z II

After the first application, *nos*Z II gene copy numbers increased in the first 15 days and then decreased (Fig. 4.21). The changing trend of *nos*Z II gene copy numbers after the second application was similar to that of *nir*S gene copy numbers described above. There was no significant difference in *nos*Z II gene copy numbers among control, CW and UE treatments (P>0.05); however, the application of TE resulted in higher copy numbers of *nos*Z II than the other treatments (P<0.05).

General Agrobacteria

After the first application, gene copy numbers of general agrobacteria reached their maximum values and declined in the following three months (Fig. 4.22). After the second application, the general agrobacteria copy numbers increased slightly in the first two months and then decreased. There was no significant difference in general agrobacteria copy numbers among the four different treatments (P>0.05).

Fungi

Gene copy numbers of fungi from the four different treatments increased slightly in the first two months after the first application and then decreased (Fig. 4.23). The changing patterns of fungi gene copy numbers after the second application were similar to those of *nir*S gene copy numbers described above. There was no significant difference in fungi gene copy numbers among control, CW and UE treatments (P>0.05); however, the application of TE resulted in higher copy numbers of fungi compared with the other treatments (P<0.05).

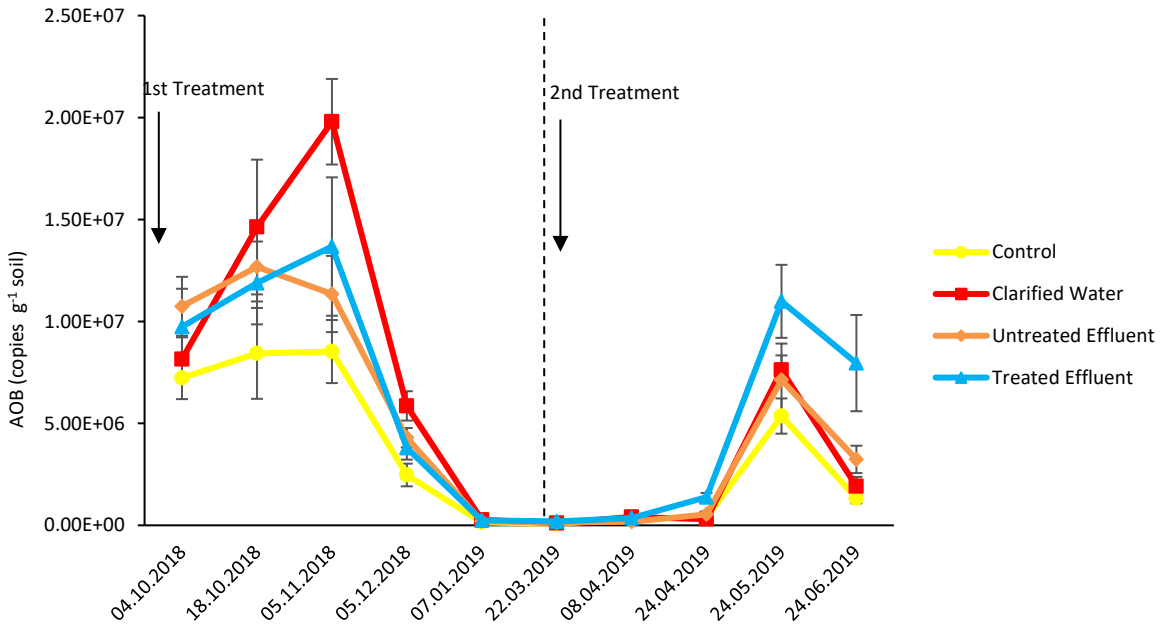

Figure 4.16 AOB *amo*A gene abundance in the soil. The error bars represent the standard error of the mean (n=6).

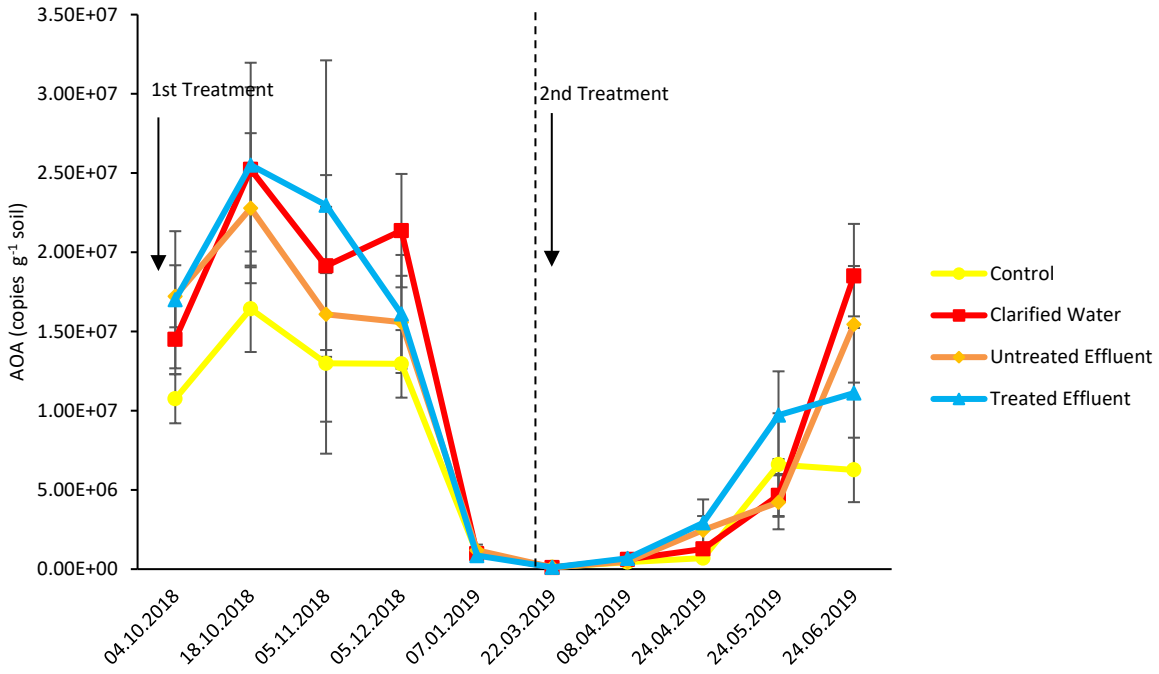

Figure 4.17 AOA *amo*A gene abundance in the soil. The error bars represent the standard error of the mean (n=6).

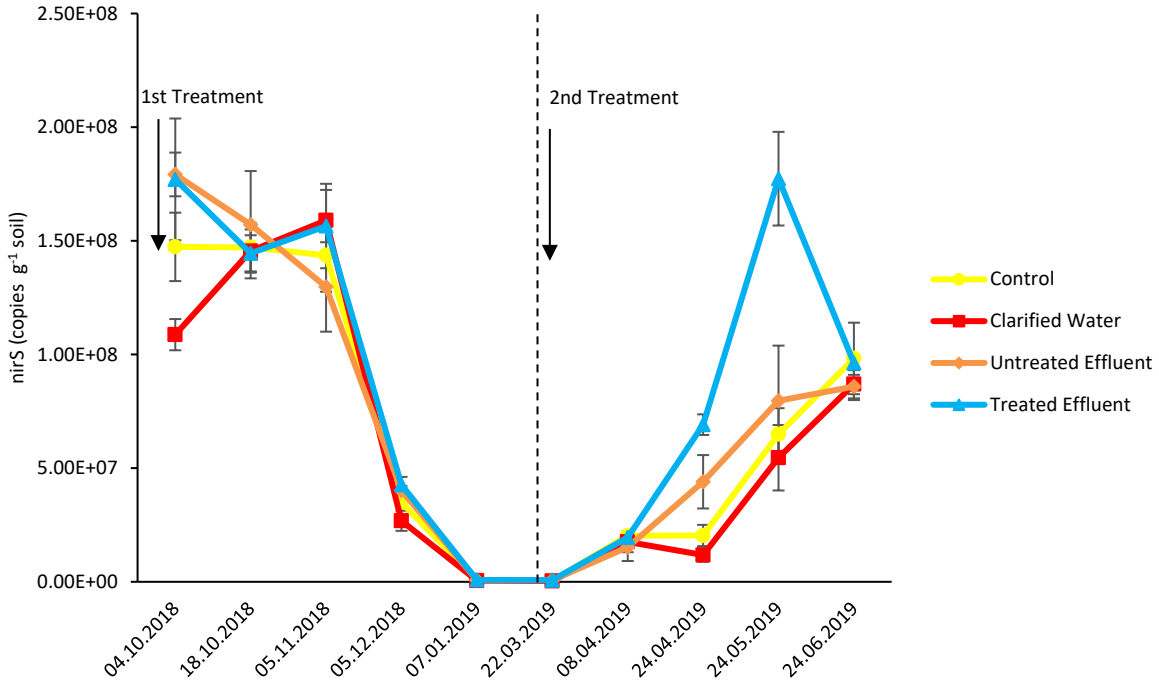

Figure 4.18 *nir*S gene abundance in the soil. The error bars represent the standard error of the mean (n=6).

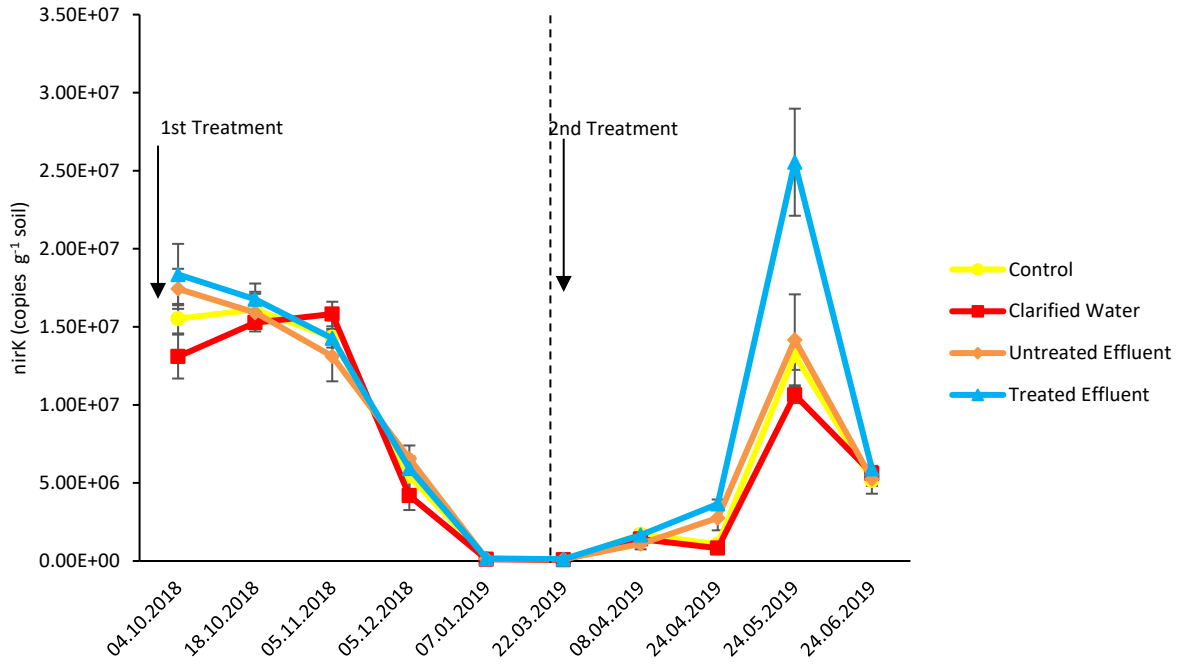

Figure 4.19 *nir*K gene abundance in the soil. The error bars represent the standard error of the mean (n=6).

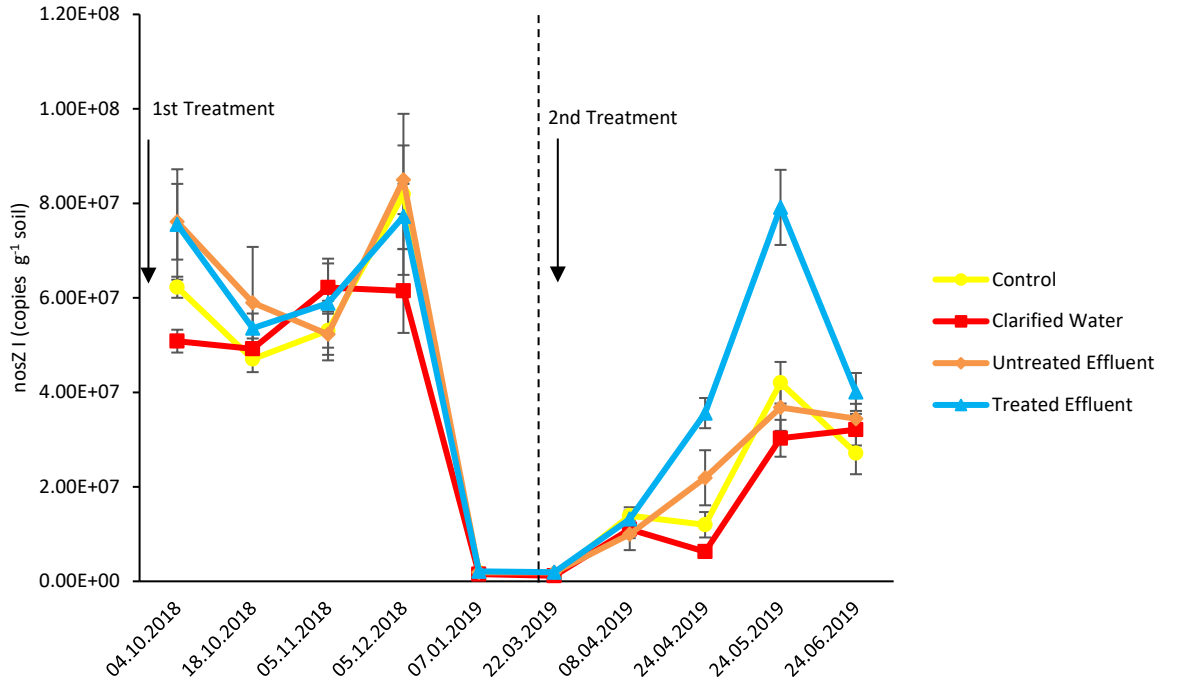

Figure 4.20 *nos*Z I gene abundance in soil. The error bars represent the standard error of the mean (n=6).

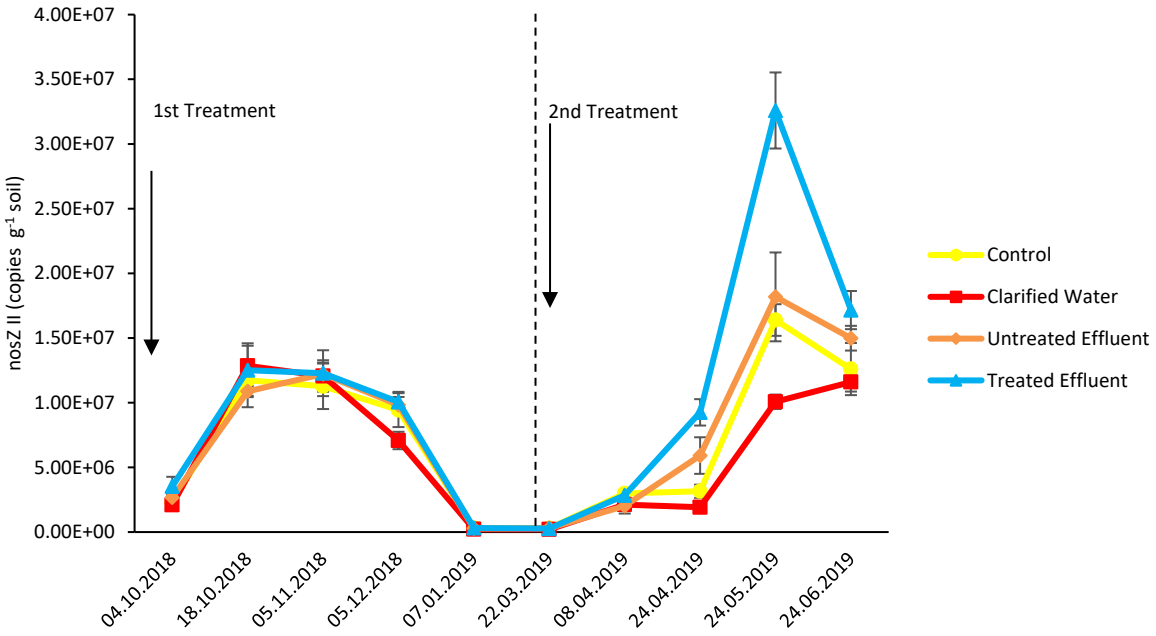

Figure 4.21 *nos*Z II gene abundance in soil. The error bars represent the standard error of the mean (n=6).

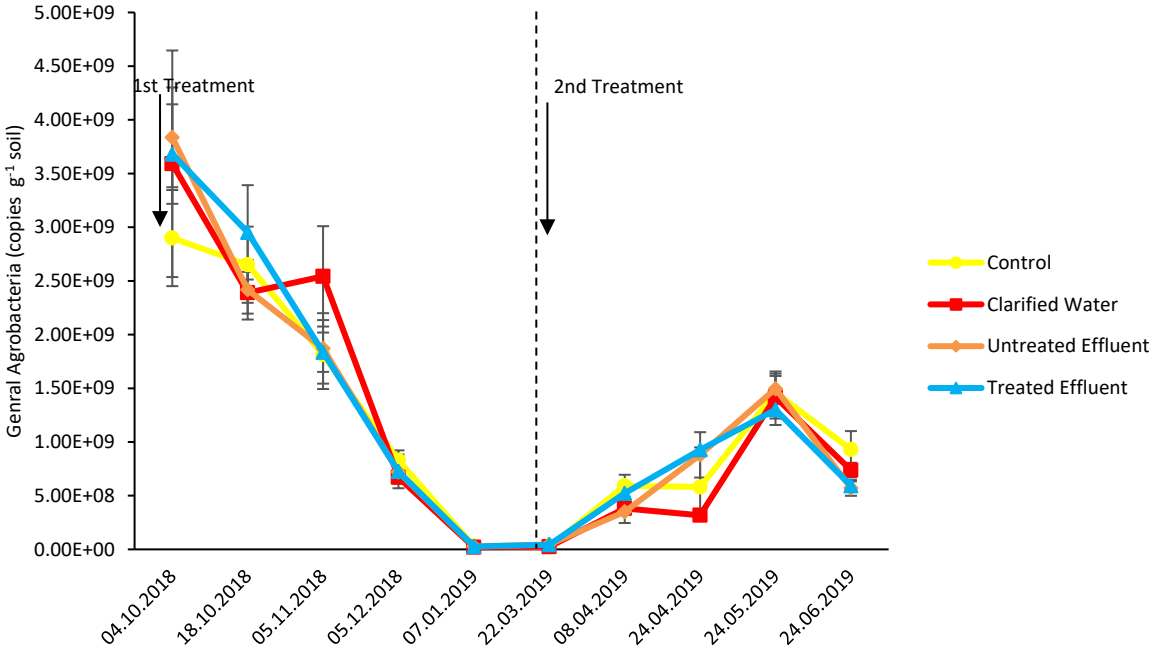

Figure 4.22 General agrobacteria gene abundance in the soil. The error bars represent the standard error of the mean (n=6).

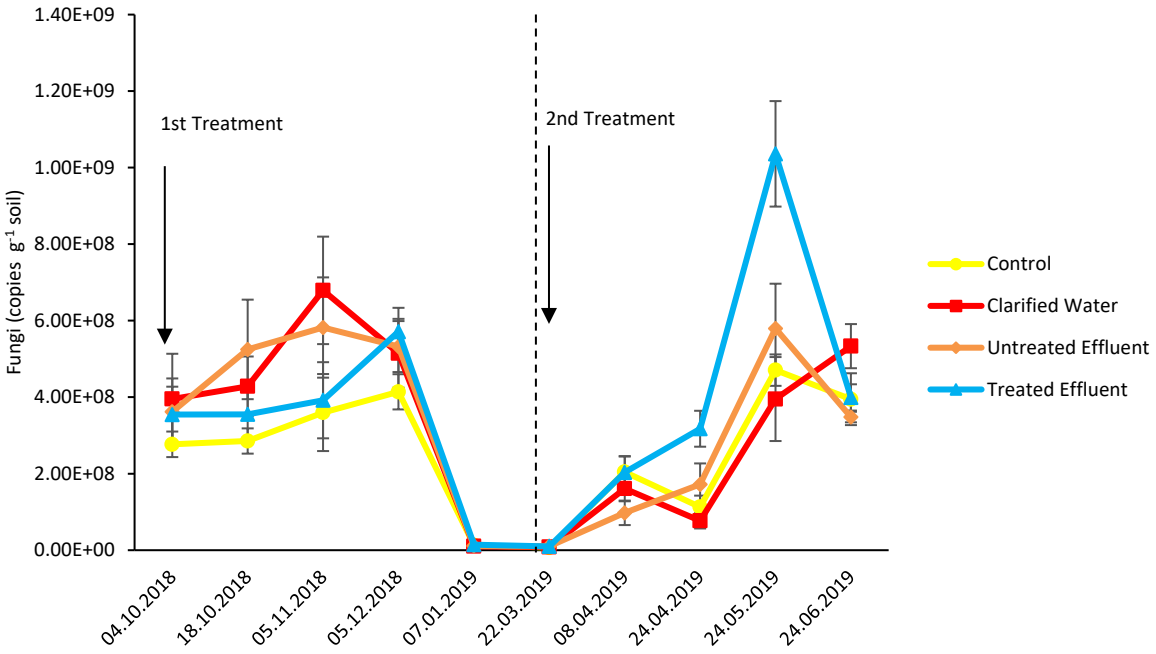

Figure 4.23 Fungi gene abundance in the soil. The error bars represent the standard error of the mean (n=6).

4.3.3 Plant

N

The concentrations of N in the pasture from the four treatments were generally similar for each harvest (Fig. 4.24a). The application of all three effluent treatments resulted in a greater amount of total N uptake compared to the control ($P<0.05$) (Fig. 4.24b).

P

The concentrations of P were similar in the four harvests (Fig. 4.25a). The P concentration in the control of each harvest was slightly higher than those in the other three treatments, which were very similar. Thus, the average P concentration in the control was significantly higher than those in the CW, TE and UE treatments in the four treatments ($P<0.05$). There was no significant difference in total P uptake of the four harvests between control and CW treatment ($P>0.05$). However, the total P uptakes in the UE and TE treatments were higher than the other treatment ($P<0.05$) (Fig. 4.25b).

K

The average K concentration in the control was significantly lower than those in the CW, TE and UE treatments in the four harvests ($P<0.05$). The total K uptakes over the four harvests in the CW, TE and UE treatments were significantly higher than that in the control ($P<0.05$) (Fig. 4.26b).

S

The concentration of S in the control was significantly lower than those in the CW, TE and UE treatments in the last three harvests ($P<0.05$) (Fig. 4.27a). The total S uptakes over the four harvests in the CW, TE and UE treatments were significantly higher than that in the control ($P<0.05$) (Fig. 4.27b).

Ca

The Ca concentration in the control was significantly higher than those from the CW, TE and UE treatments in the four harvests (Fig. 4.28a) ($P<0.05$). The total Ca uptake over the four harvests in the control was higher than those from CW, TE and UE treatments (Fig. 4.28b).

Mg

The concentration of Mg in the four treatments remained steady in the first three harvests and increased slightly in the fourth harvest (Fig. 4.29a). The Mg concentration in the control was significantly higher than those in the CW, TE and UE treatments in the four harvests ($P<0.05$). There was no significant difference in the total Mg uptake among the different treatments ($P>0.05$) (Fig. 4.29b).

Na

The concentration of Na in the control was significantly higher than those in the CW, TE and UE treatments in the four harvests ($P<0.05$). The Na concentration in the CW was the lowest in the four treatments. There was no significant difference in the total Na uptake of the four harvests between the control, UE and TE treatments ($P>0.05$) (Fig. 4.30b). However, the total Na uptake in CW was significantly lower than those in the other treatments.

Fe

The concentrations of Fe were generally similar in the four treatments, except in the second harvest where it was significantly higher in the treated effluent than in the other treatments ($P<0.05$, Fig. 4.31a). There was no significant difference in the total Fe uptake of the four harvests between the control and the CW and UE treatments ($P>0.05$) (Fig. 4.31b). However, the total Fe uptake in TE was significantly higher than the other treatments ($P<0.05$).

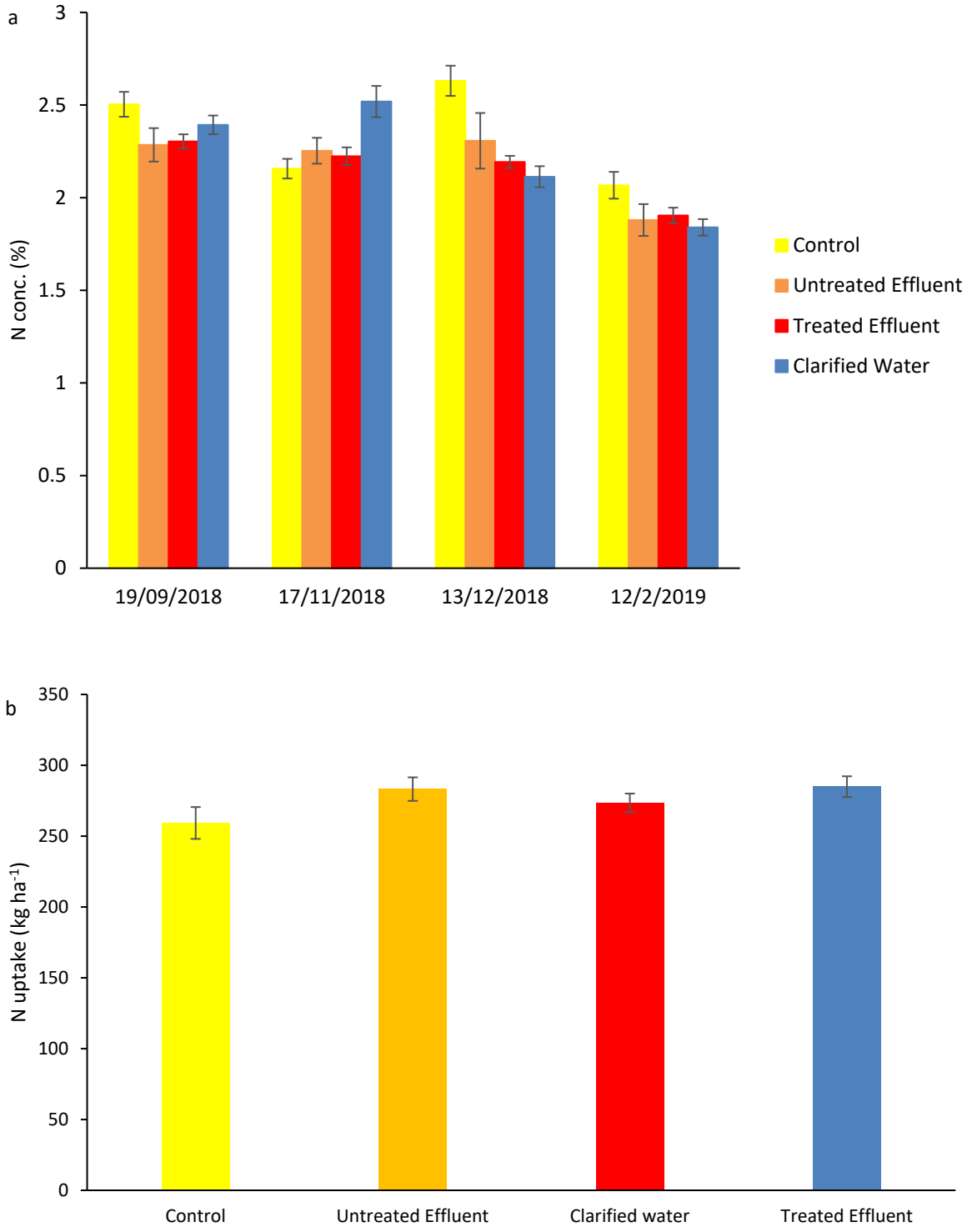

Figure 4.24 N in the pasture. (a): average N concentration over the four harvests; (b): total N uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

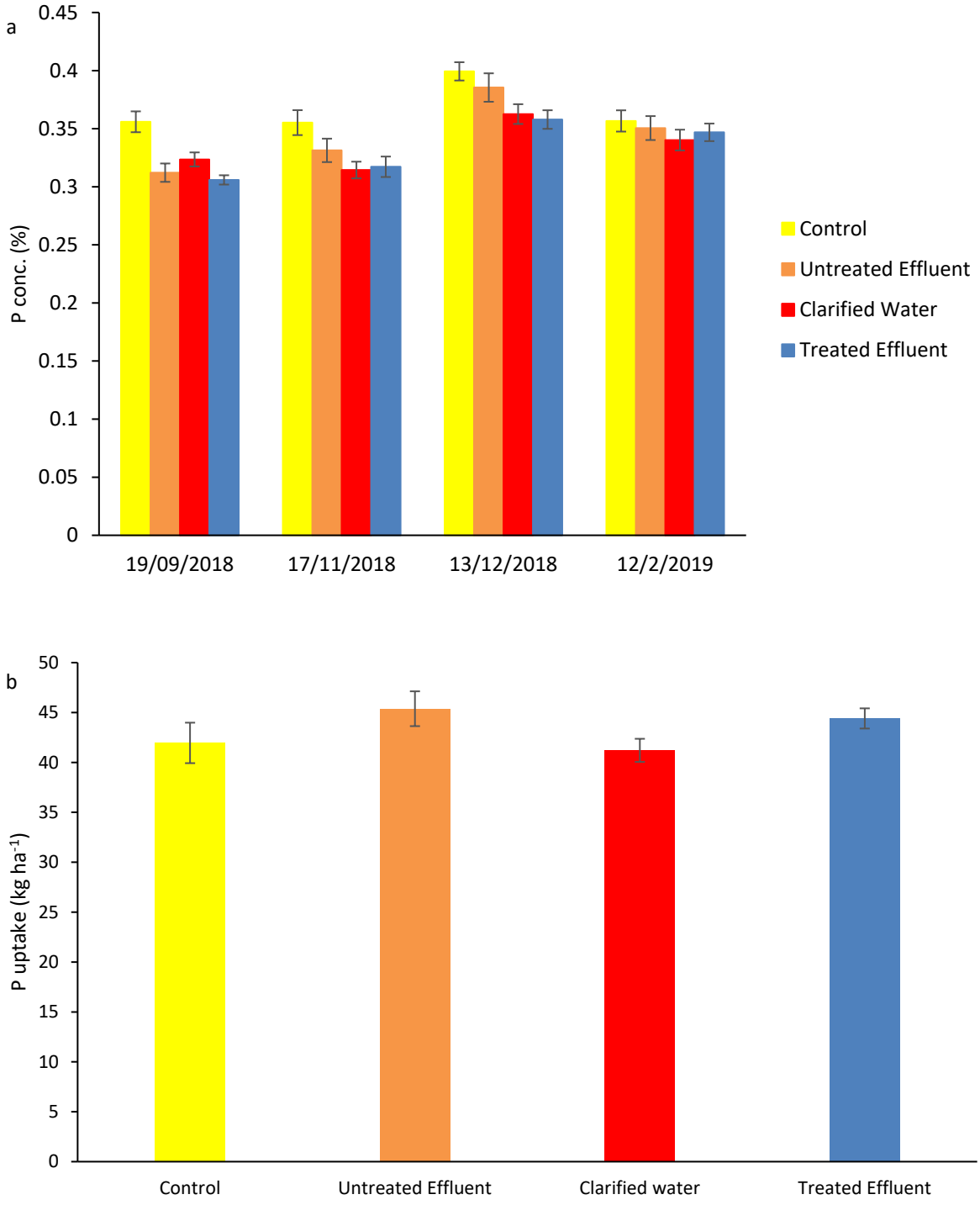

Figure 4.25 P in the pasture. (a): average P concentration over the four harvests; (b): total P uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

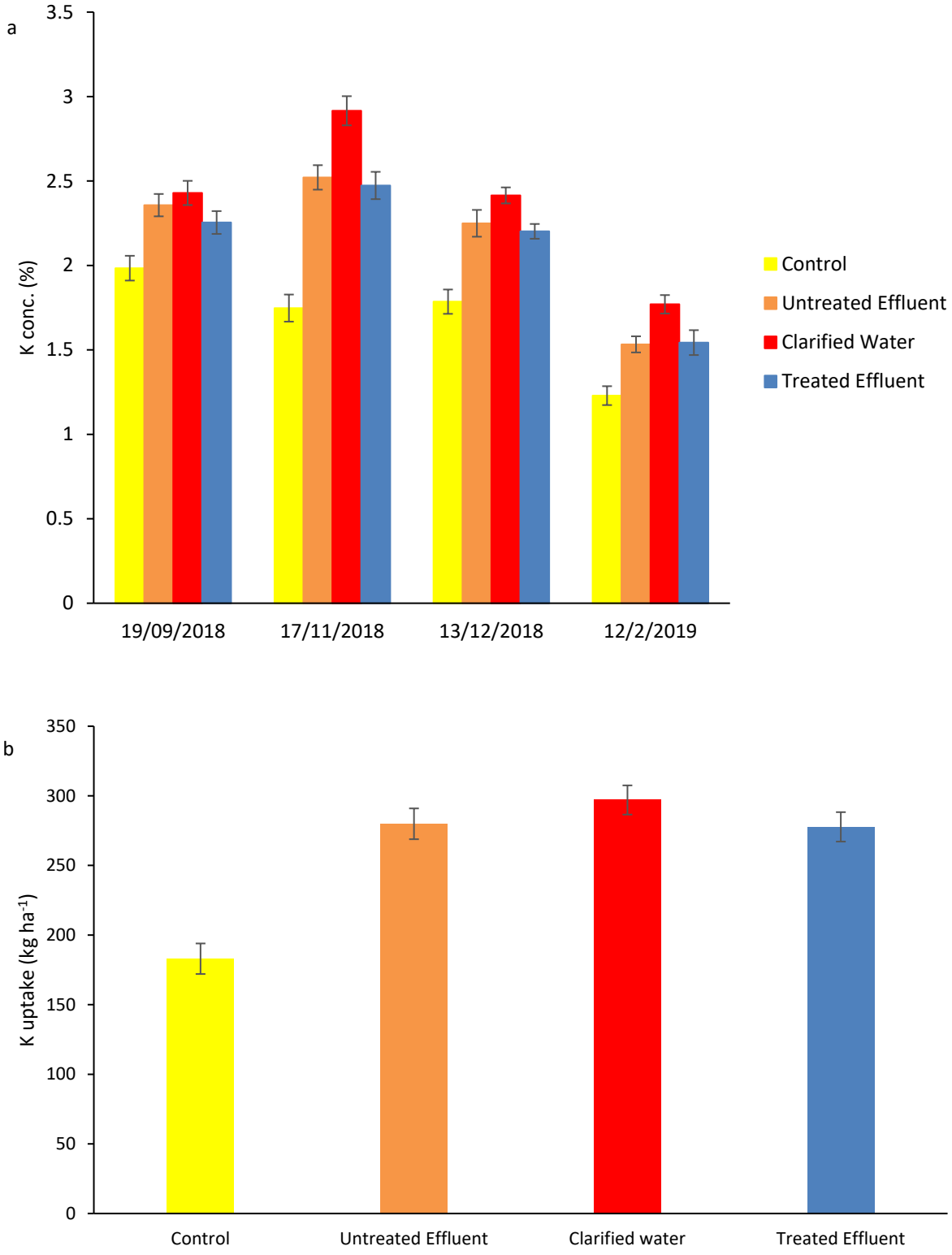

Figure 4.26 K in the pasture. (a): average K concentration over the four harvests; (b): total K uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

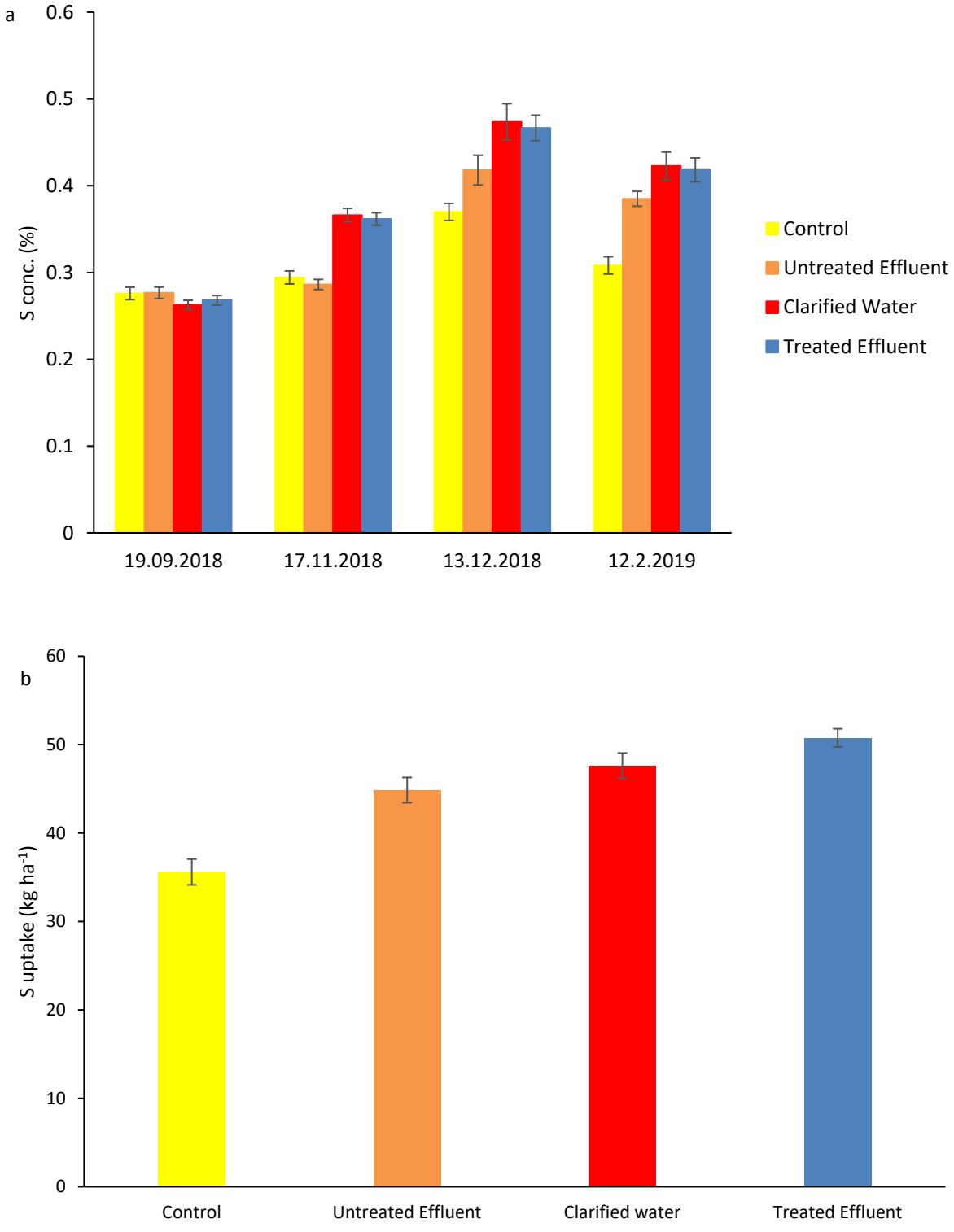

Figure 4.27 S in the pasture. (a): average S concentration over the four harvests; (b): total S uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

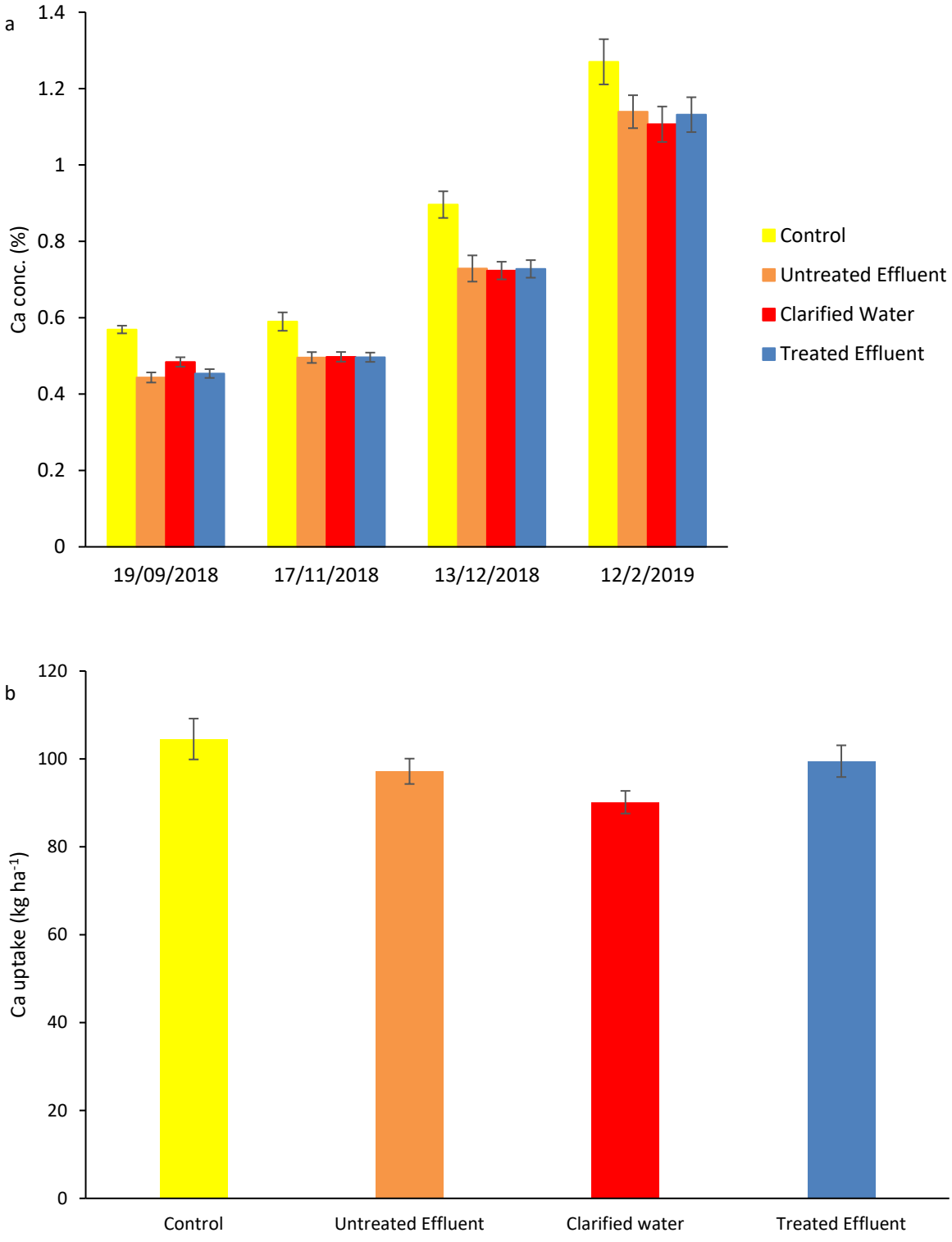

Figure 4.28 Ca in the pasture. (a): average Ca concentration over the four harvests; (b): total Ca uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

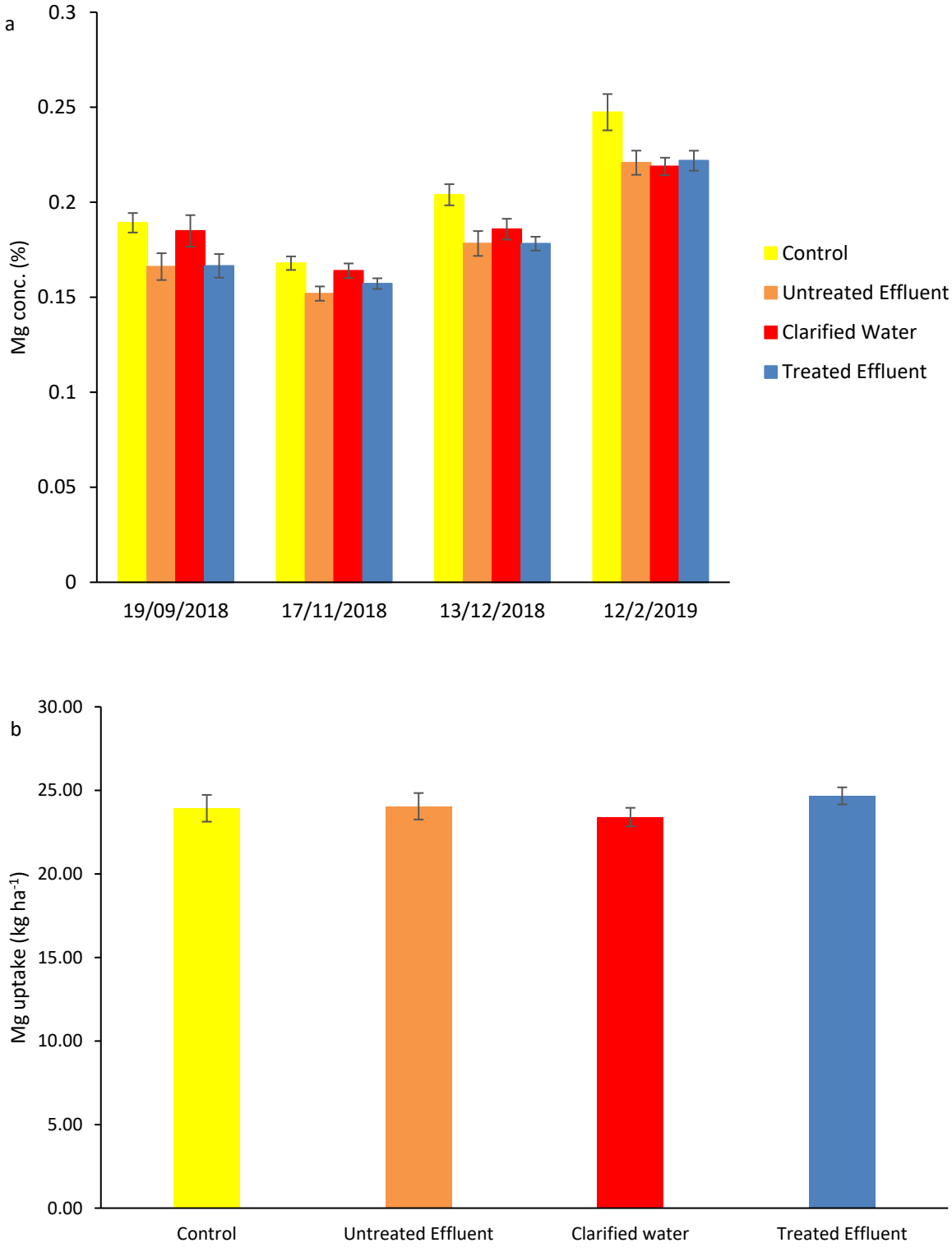

Figure 4.29 Mg in the pasture. (a): average Mg concentration over the four harvests; (b): total Mg uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

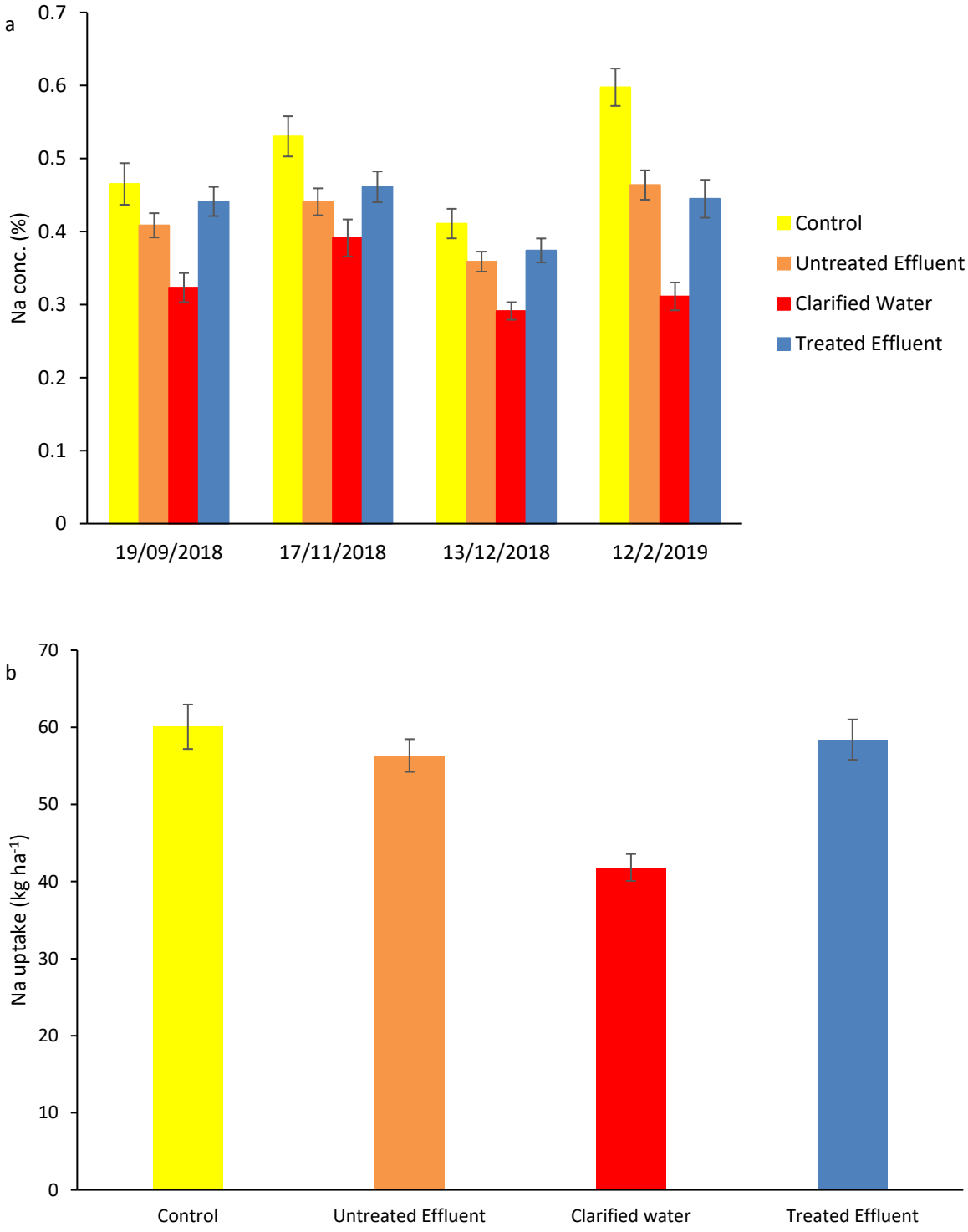

Figure 4.30 Na in the pasture. (a): average Na concentration over the four harvests; (b): total Na uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

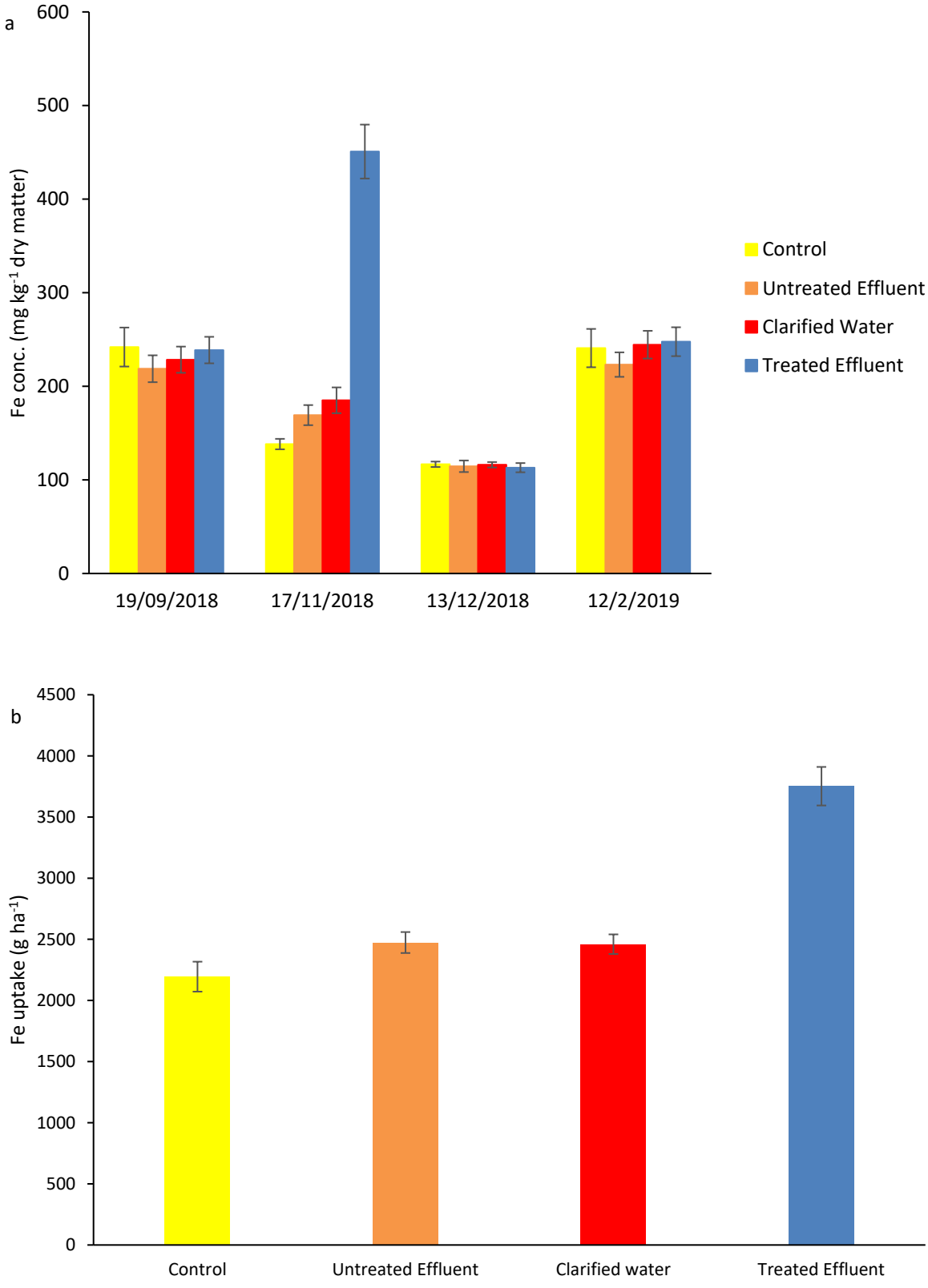

Figure 4.31 Fe in the pasture. (a): average Fe concentration over the four harvests; (b): total Fe uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

Al

The concentration of Al decreased sharply after application and increased in the fourth harvest (Fig. 4.32a). There was no significant difference between the different effluent treatments ($P>0.05$). There was no significant difference in the total Al uptake in the four harvests among all effluent treatments ($P>0.05$) (Fig. 4.32b).

B

The B concentration in the control was significantly higher than those in the CW, TE and UE treatments in the four harvests ($P<0.05$) (Fig. 4.33a). There was no significant difference in the total B uptake of the four harvests among CW, TE and UE treatments ($P>0.05$) (Fig. 4.33b). The total B uptake in the control was significantly higher than the other effluent treatments.

Cu

There was no significant difference in Cu concertation among the control, CW and TE treatments ($P>0.05$). The Cu concentration in the UE was significantly lower than that in the control in the four harvests ($P<0.05$). The application of the effluent treatments resulted in higher total Cu uptakes of the four harvest than that in the control (Fig. 4.34b). There was no significant difference in the total Cu uptake among the CW, TE and UE treatment ($P>0.05$).

Mn

The concentration of Mn in the pasture decreased slightly in the four harvests (Fig. 4.35a). The Mn concentration in the CW was significantly higher than the other three treatments ($P<0.05$). There was no significant difference among the control, UE and TE treatments($P>0.05$). The application of the three effluent treatments resulted in higher total Mn uptake of the four harvests than that in the control, and the total Mn uptake in the CW was the highest (Fig. 4.35b).

Mo

The application of the effluent treatments resulted in lower Mo concentrations in the plants (Fig. 4.36a). The Mo concentration in the control was significantly higher than that from the other three treatments ($P<0.05$). There was no significant difference in the total Mo uptake of the four harvests between the control, UE and TE treatments ($P>0.05$) while the Mo uptake in the CW was significantly lower than that in the control (Fig. 4.36b).

Zn

The Zn concentration from the four treatments was similar in the four harvests (Fig. 4.37a). There was no significant difference in Zn concentration among the different effluent treatments ($P>0.05$).

The application of the three effluent treatments resulted in higher total Zn uptake of the four harvests than that in the control, and the total Zn uptake in the UE was the highest (Fig. 4.37b).

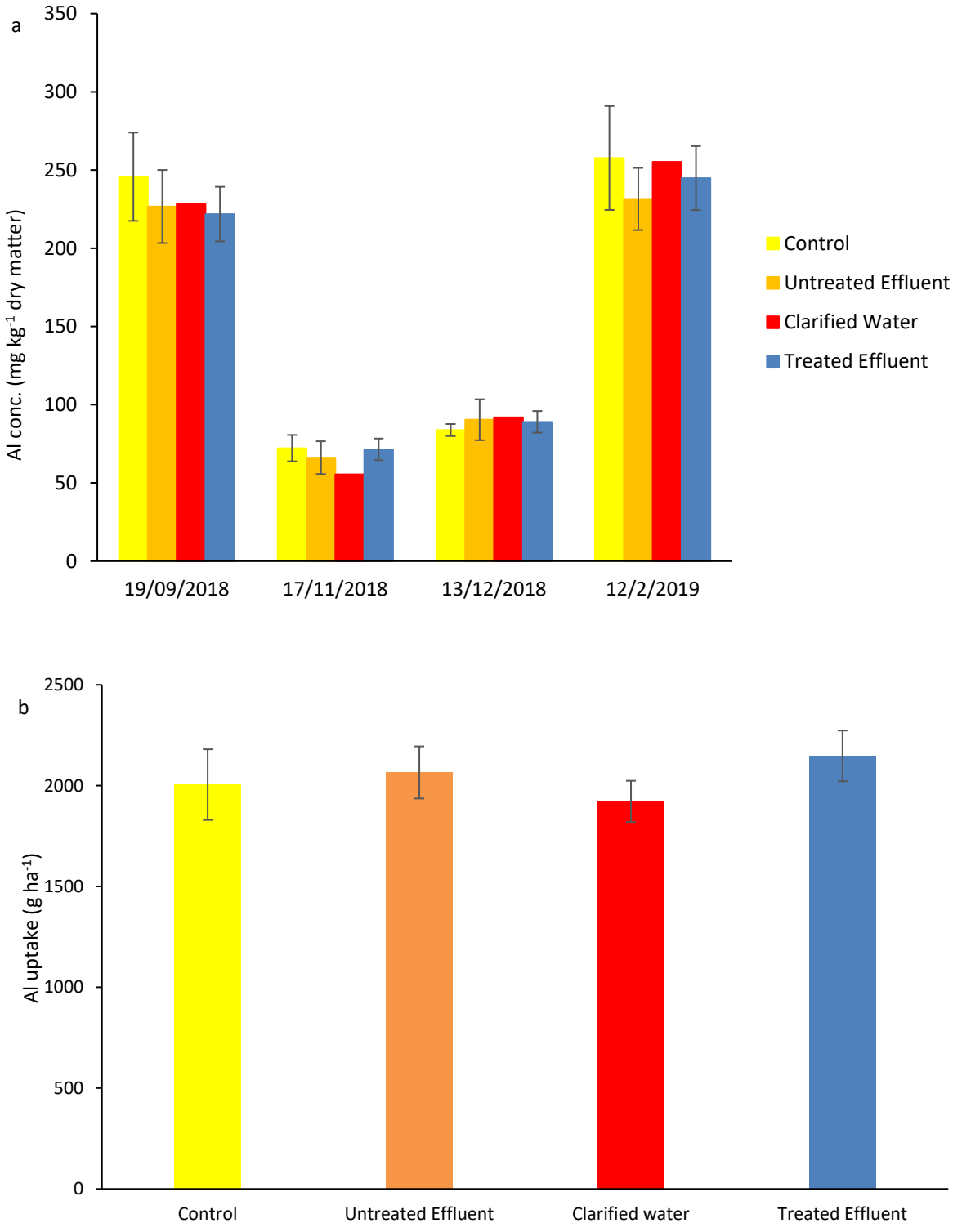

Figure 4.32 Al in the pasture. (a): average Al concentration over the four harvests; (b): total Al uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

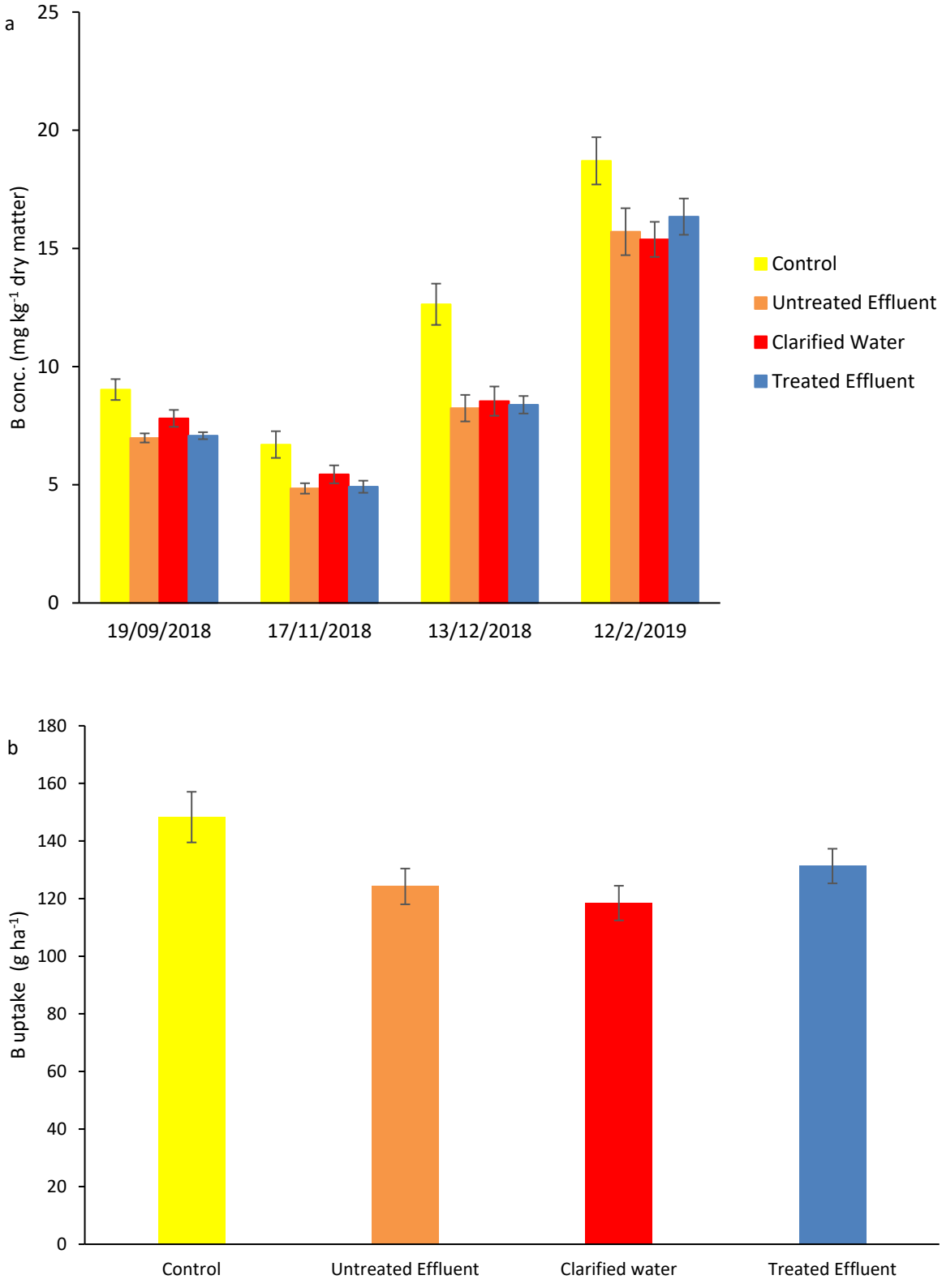

Figure 4.33 B in the pasture. (a): average B concentration over the four harvests; (b): total B uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

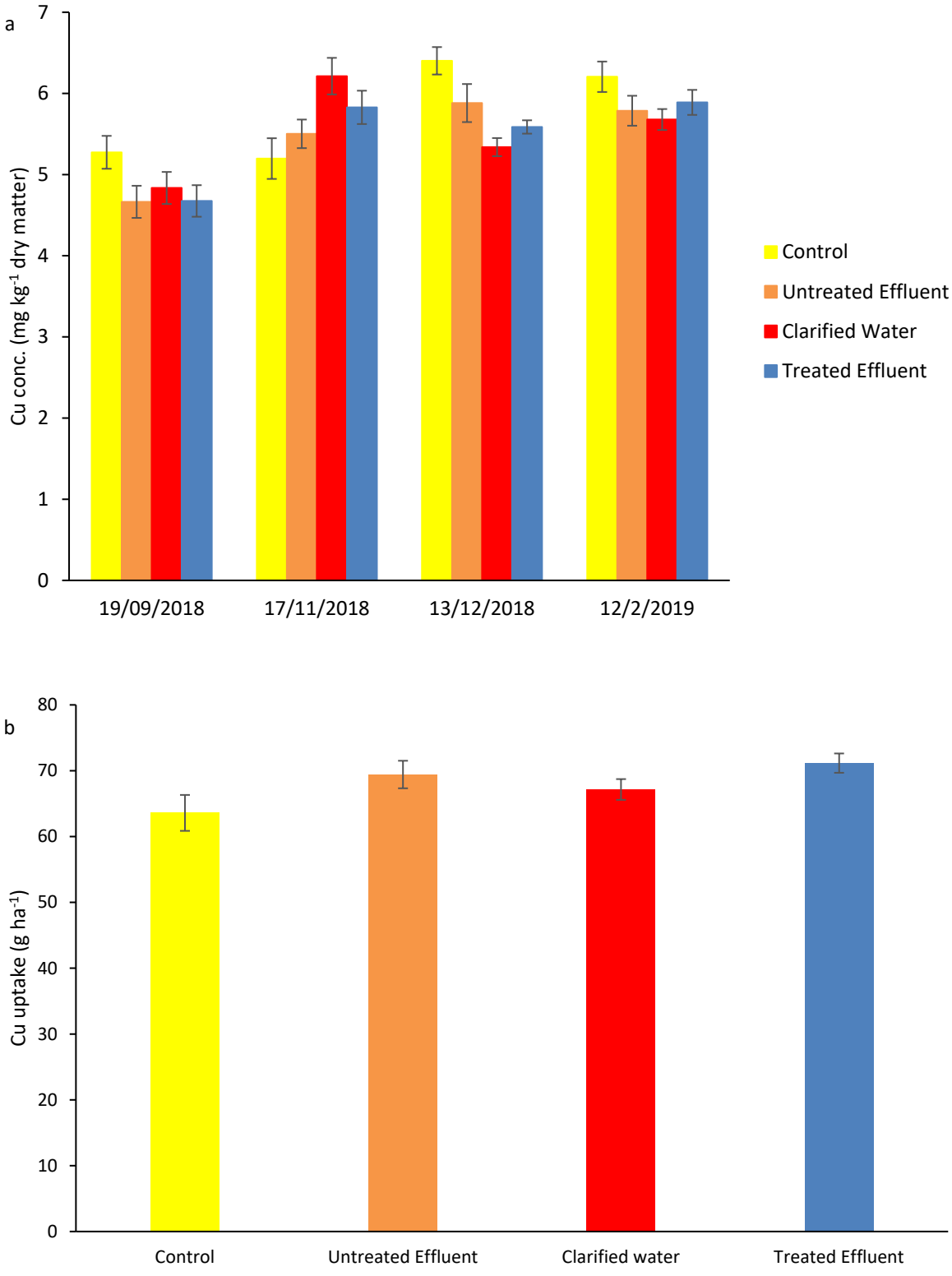

Figure 4.34 Cu in the pasture. (a): average Cu concentration over the four harvests; (b): total Cu uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

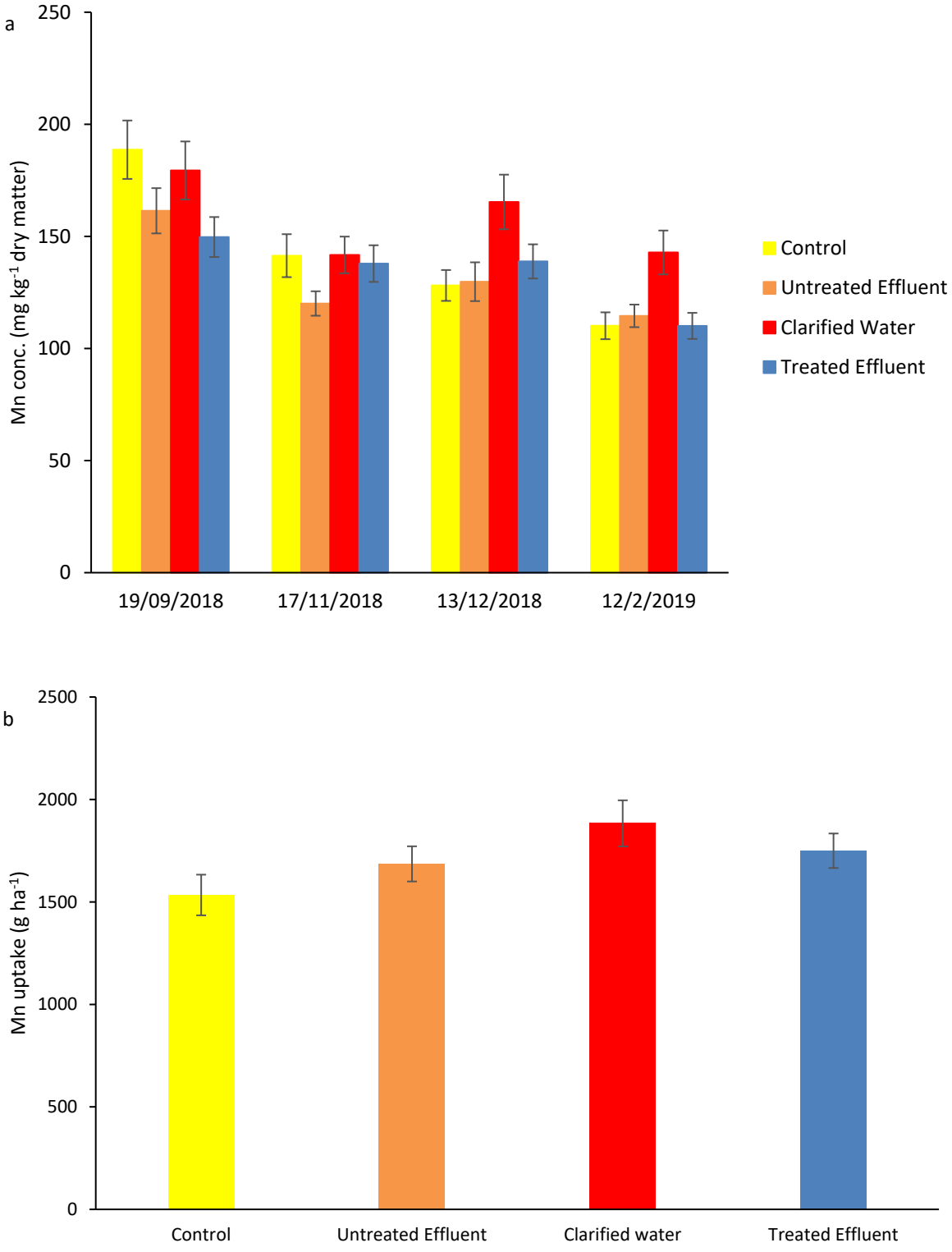

Figure 4.35 Mn in the pasture. (a): average Mn concentration over the four harvests; (b): total Mn uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

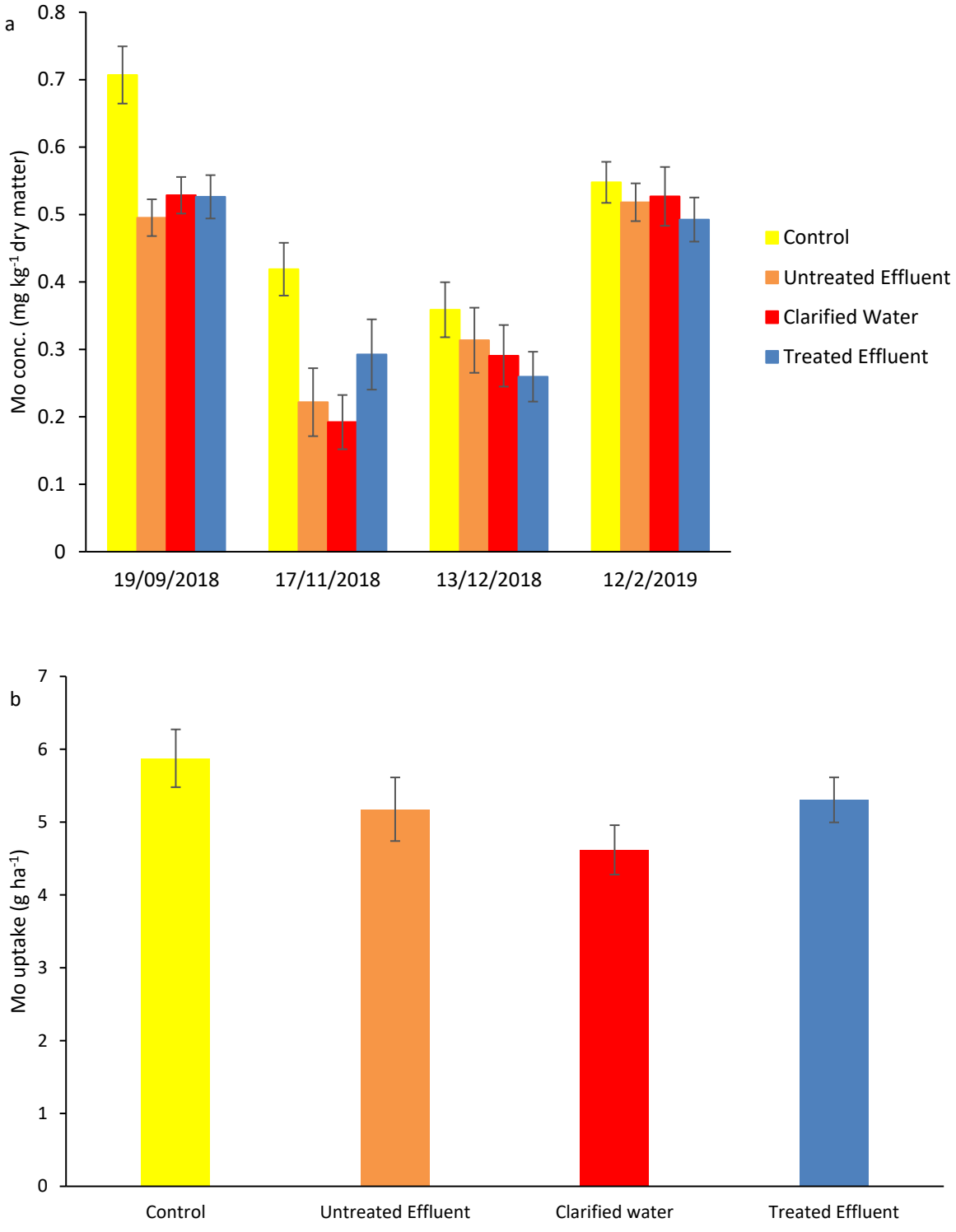

Figure 4.36 Mo in the pasture. (a): average Mo concentration over the four harvests; (b): total Mo uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

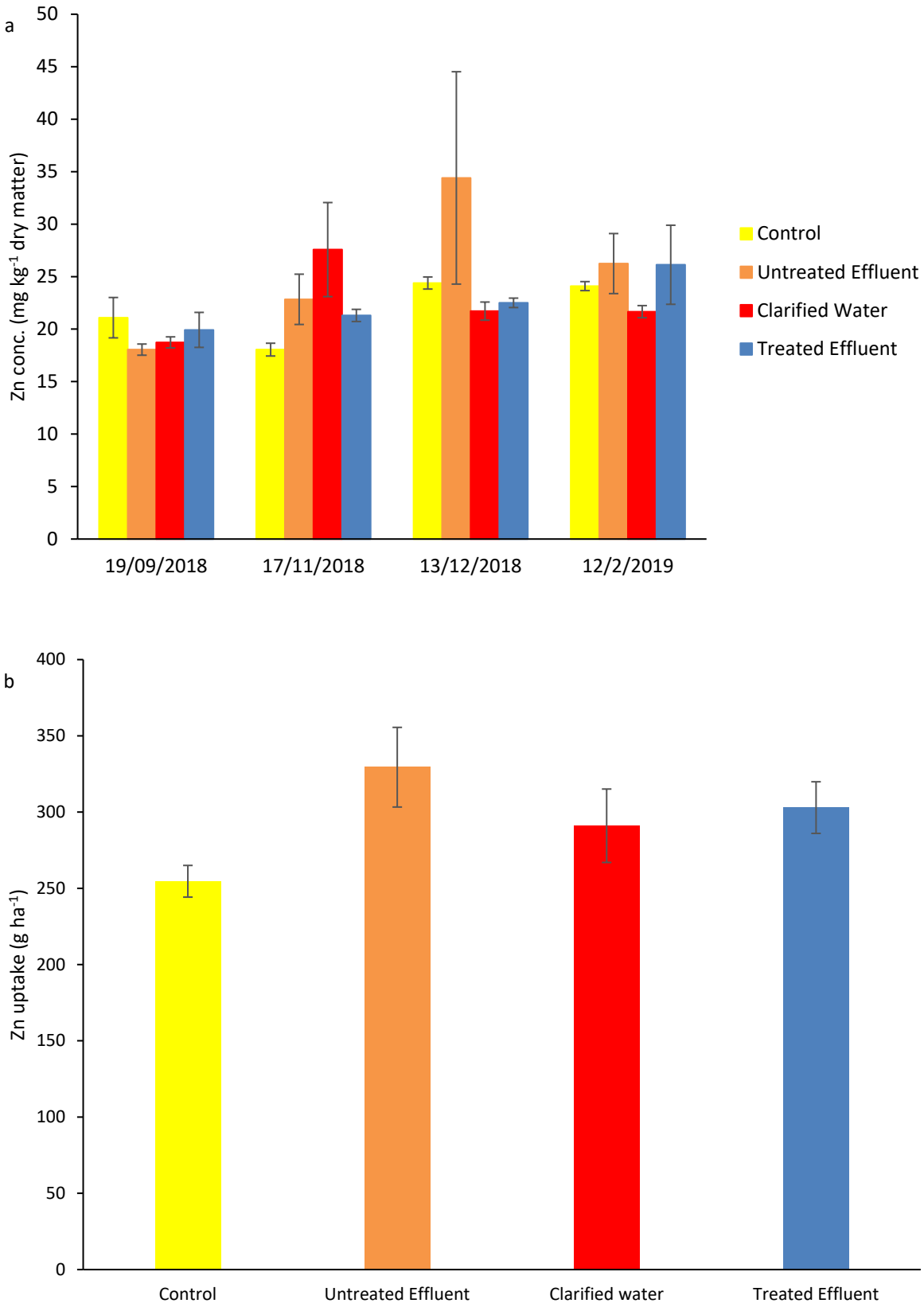

Figure 4.37 Zn in the pasture. (a): average Zn concentration over the four harvests; (b): total Zn uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

As

The concentration of As decreased sharply with time after application (Fig. 4.38a). There was no significant difference in the As concentration among the different treatments (P>0.05). There was no significant difference in the total As uptake of the four harvests among all the effluent treatments (P>0.05) (Fig. 4.38b).

Cd

The concentration of Cd remained steady at a low level and increased in the fourth harvest (Fig. 4.39a). There was no significant difference in the Cd concentration among the different treatments (P>0.05). There was no significant difference in the total Cd uptake of the four harvests among all the effluent treatments (P>0.05) (Fig. 4.39b).

Cr

There was no significant difference in the Cr concentration among the different treatments (P>0.05) (Fig. 4.40a). There was no significant difference in the total Cr uptake of the four harvests among all the effluent treatments (P>0.05) (Fig. 4.40b).

Ni

There was no significant difference in Ni concentration among the different treatments (P>0.05) (Fig. 4.41a). There was no significant difference in the total Ni uptake of the four harvests among all the effluent treatments (P>0.05) (Fig. 4.41b).

Pb

There was no significant difference in Pb concentration among the different treatments (P>0.05) (Fig.4.42a). There was no significant difference in the total Pb uptake of the four harvests among all the effluent treatments (P>0.05) (Fig. 4.42b).

Dry Matter

The application of different effluent treatments resulted in a significant difference in the dry matter among difference treatments (P<0.05). The dry matter yields of second and fourth harvests were nearly twice those of the first and third harvest (Fig. 4.43a). Dry matter from the UE, CW and TE treatments was significantly higher than that from control in the first three harvests. The total dry matter of the four harvests from the control was significantly lower than those in the other effluent treatments (P<0.05) (Fig. 4.43b).

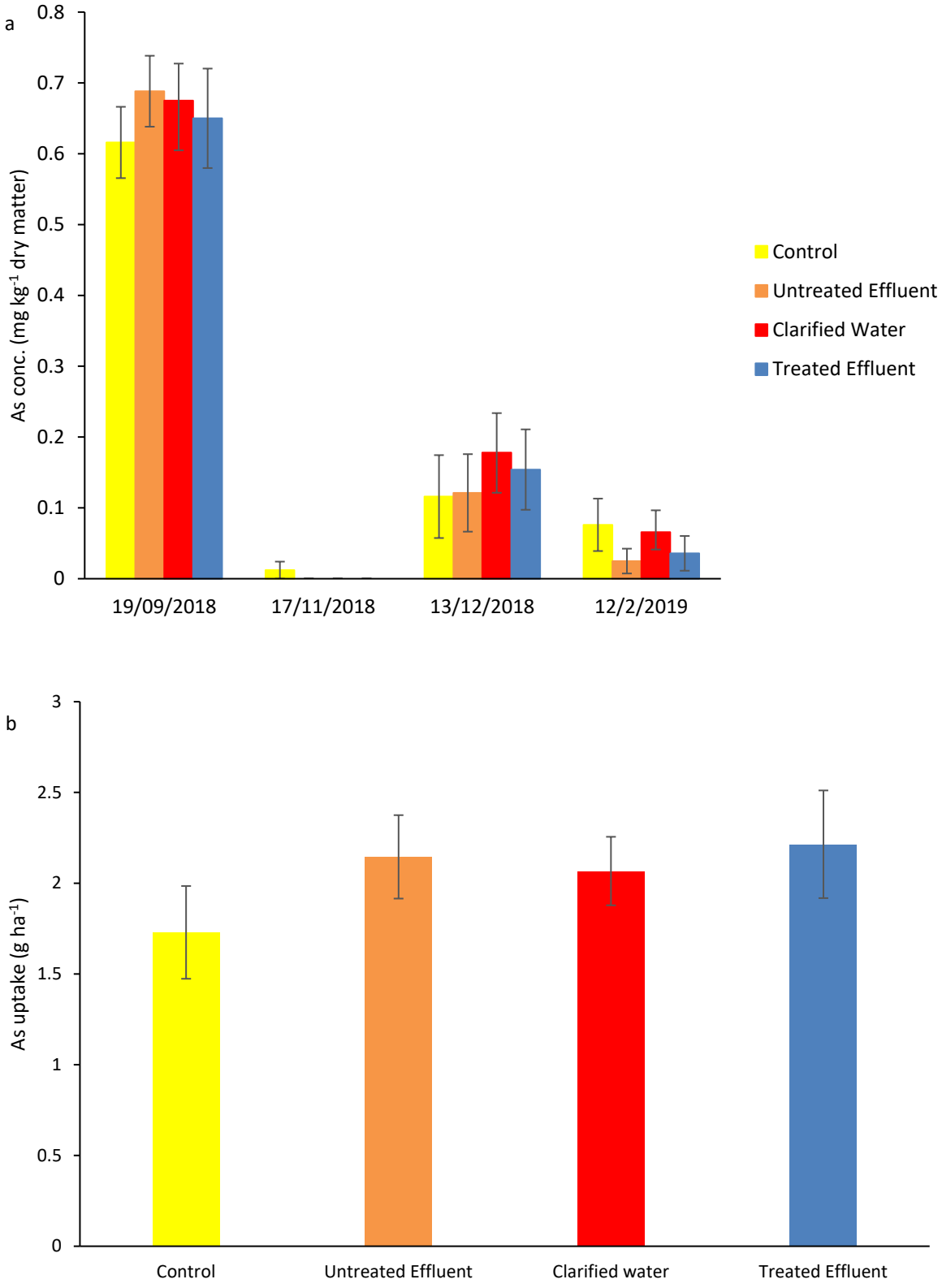

Figure 4.38 As in the pasture. (a): average As concentration over the four harvests; (b): total As uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

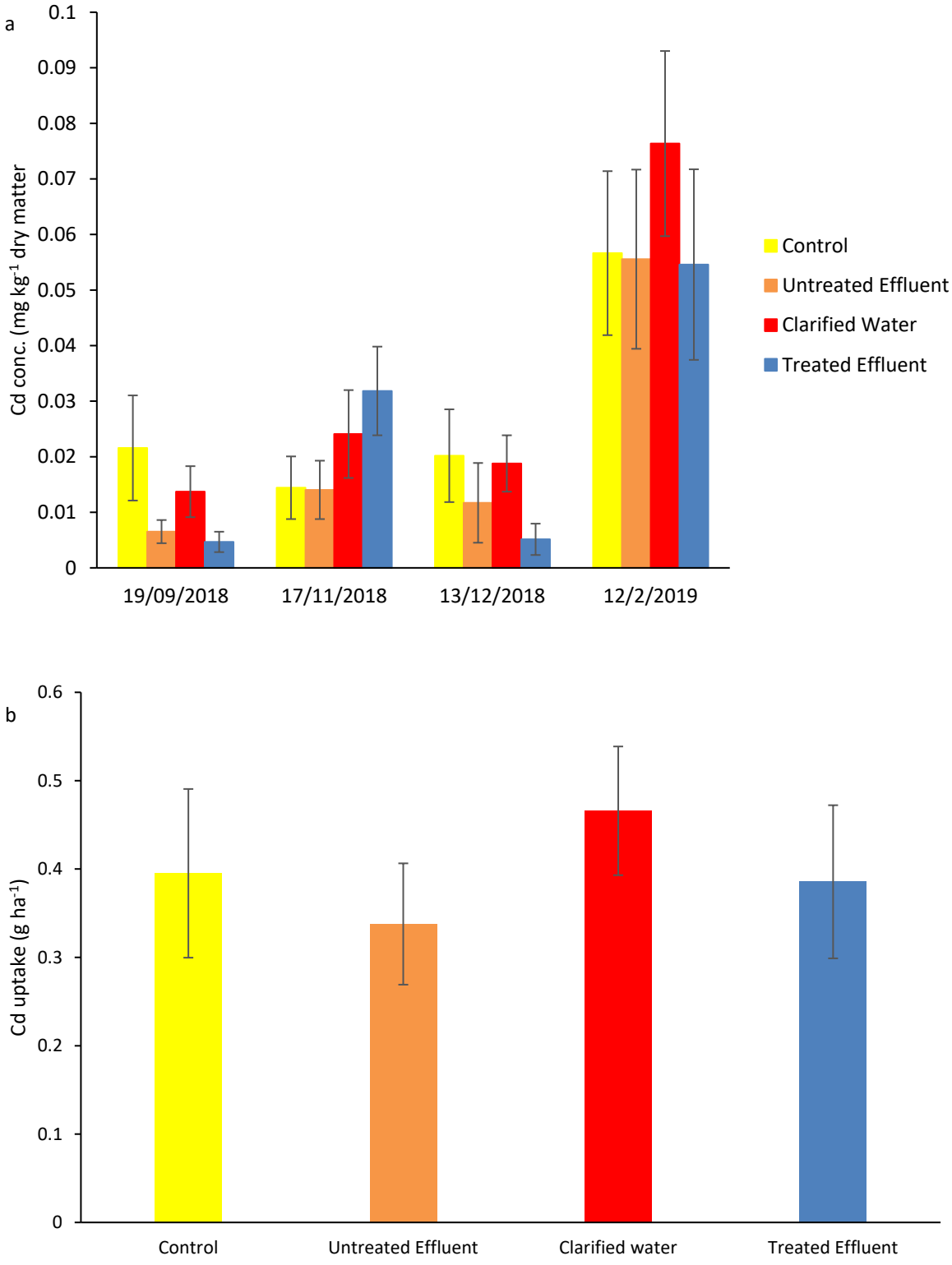

Figure 4.39 Cd in the pasture. (a): average Cd concentration over the four harvests; (b): total Cd uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

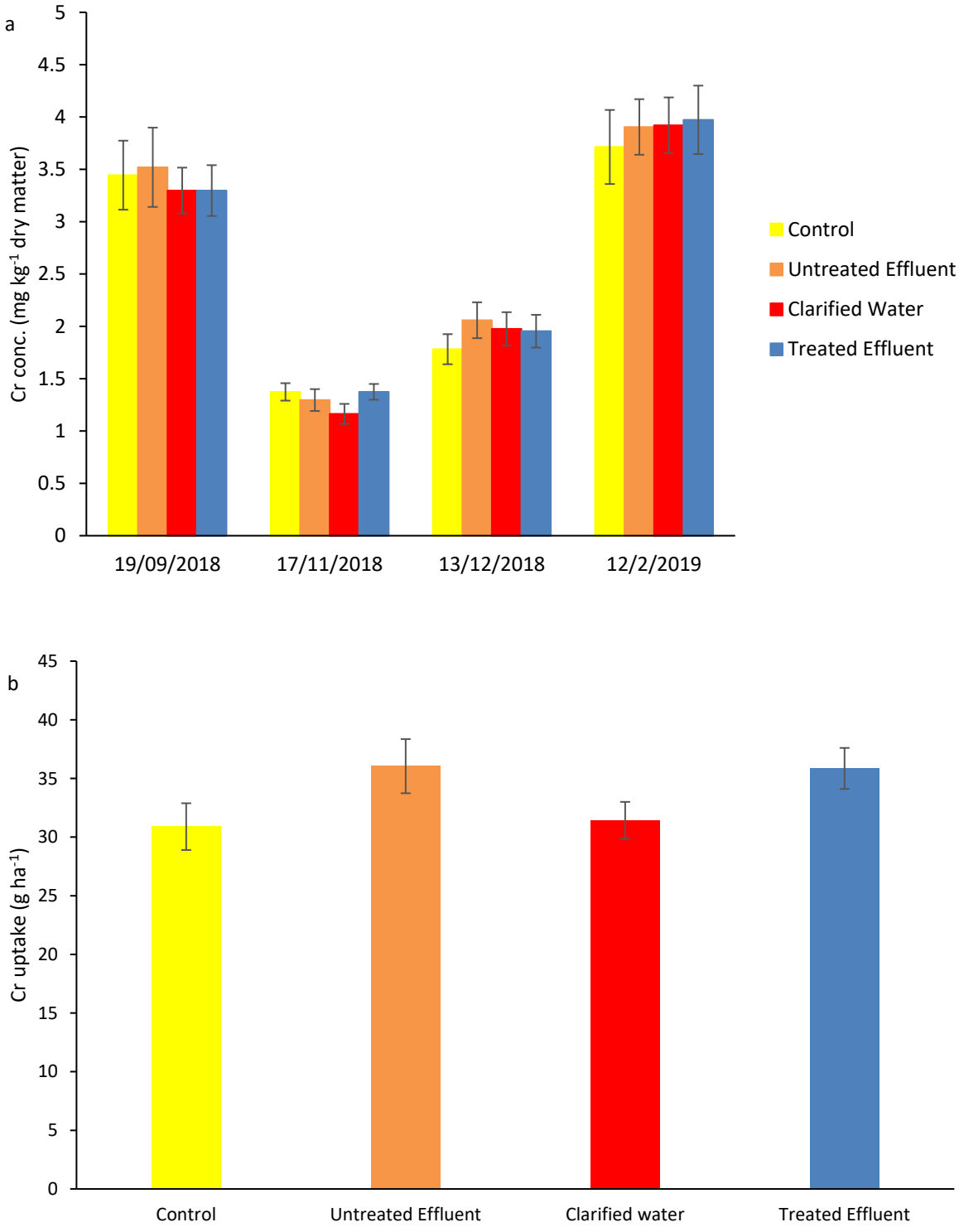

Figure 4.40 Cr in the pasture. (a): average Cr concentration over the four harvests; (b): total Cr uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

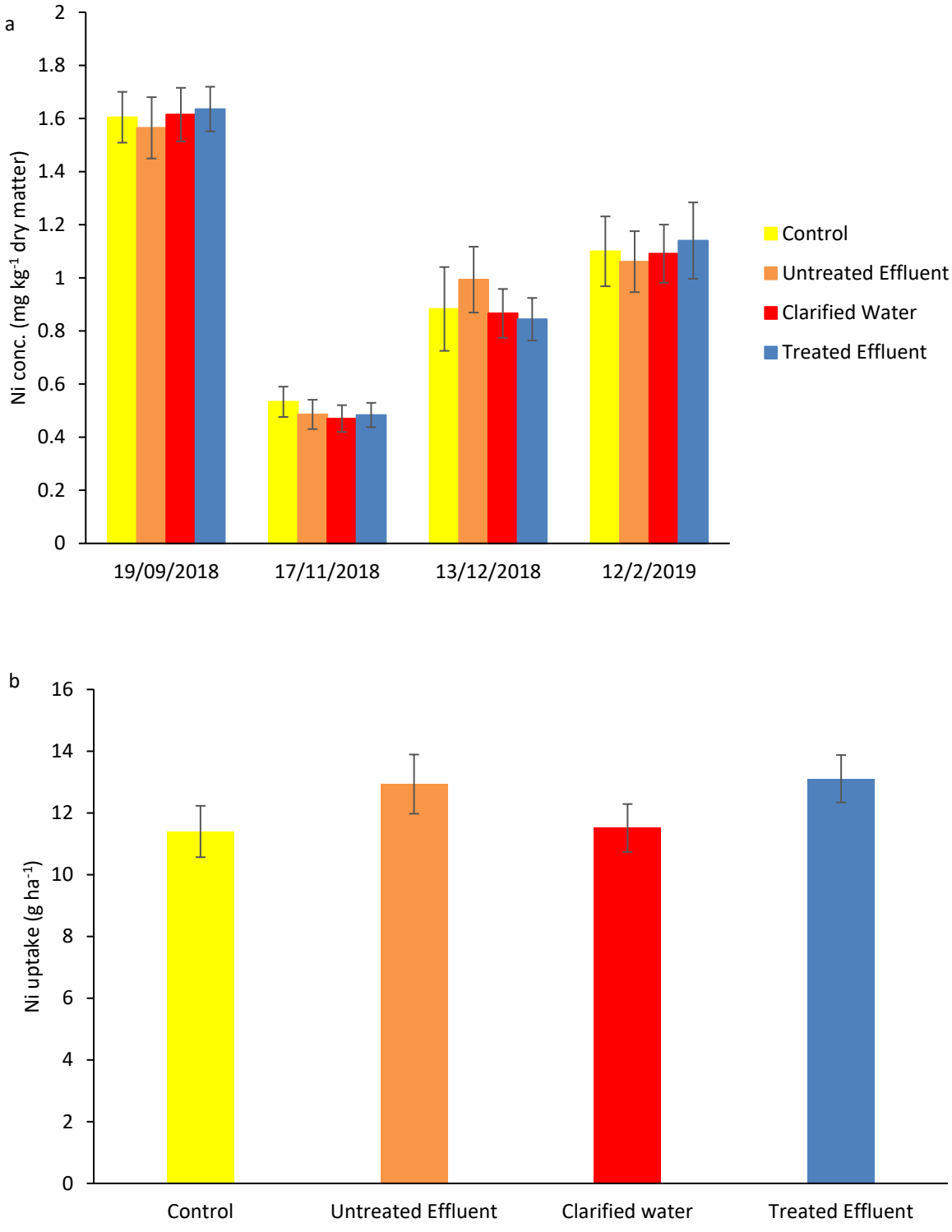

Figure 4.41 Ni in the pasture. (a): average Ni concentration over the four harvests; (b): total Ni uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

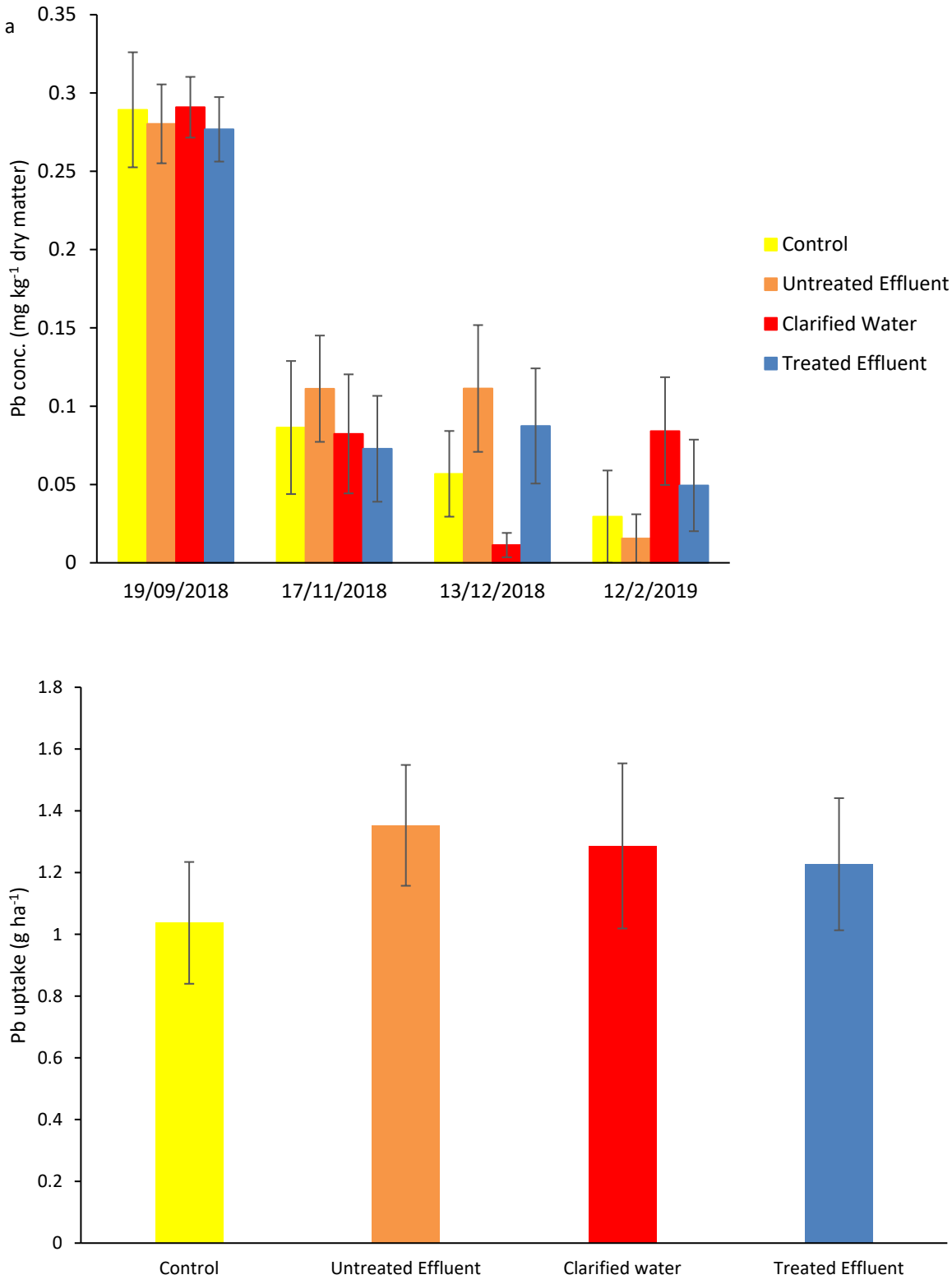

Figure 4.42 in the pasture. (a): average Pb concentration over the four harvests; (b): total Pb uptake of the four harvests. The error bars represent the standard error of the mean (n=17).

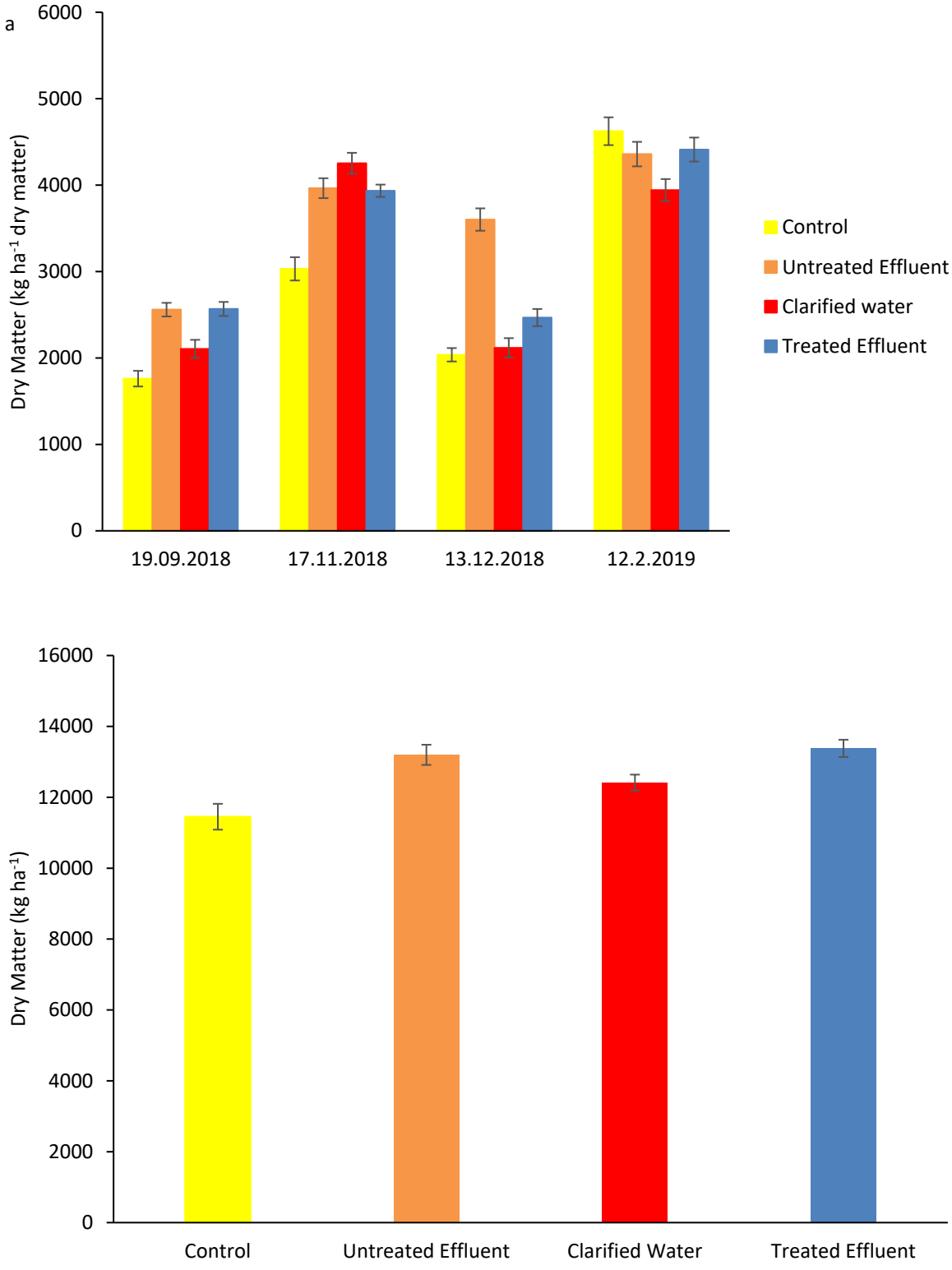

Figure 4.43 Dry matter of the pasture. (a): the amount of dry matter of each harvest; (b): Total dry matter of four harvests. The error bars represent the standard error of the mean (n=17).

4.4 Discussion

4.4.1 Effect of different effluents on soil fertility

In general, the application of farm dairy effluent is considered as an effective method to improve soil nutrient levels because the effluent contain large amounts of various nutrients(Bolan et al., 2004; Degens et al., 2000; Manono et al., 2016). The ClearTech® treatment technology separated UE into TE and CW by using PFS to cause coagulation and flocculation of the colloidal particles in the effluent (Cameron & Di, 2018). It is important to ensure that the application of TE and CW do not adversely affect soil fertility or plant growth. In this field study, results showed that the soil organic matter content, total-C and total-N were significantly increased after the application of TE compared with the other three treatments, including the UE (Fig 4.1, 4.2, and 4.3). The higher contents of total solids and total-N in TE (Table 3.1) may be the main reason resulted for these increases in the TE treatment.

Because PFS contained S, the application of TE and CW, therefore, increased soil organic S and sulphate S contents compared to the control and UE treatments (Fig 4.5 and 4.6). The higher concentrations of total-P in the TE also led to higher Olsen-P values in the soil compared with the other treatments. These results demonstrate that the application TE would have a positive effect on these soil fertility indices over the long-term. In general, CW is mainly recycled to wash the dairy milking yard, so it is not generally applied alone to the farm.

The downward trends of soil NH_4^+-N concentration after the application of the different effluents were probably a result of the nitrification process and plant uptake (Fig. 4.9). The decline in the concentrations of NO_3^--N with time was probably because of plant uptake and leaching through the soil (Fig. 4.10).

The application of the three effluents did not result in significant differences in CEC, and exchangeable Ca^{2+}, Mg^{2+}, Na^+ and K^+ in the soil (Fig. 4.11). These results again demonstrate that the application of TE or CW would not adversely affect these soil fertility indices compared with the application of UE.

4.4.2 Effect of different effluents on functional gene abundance

The growth and activity of nitrifying bacteria (AOB and AOA), which play a key role in the N cycle, can be affected by soil and environmental conditions (Di et al., 2014; Robinson et al., 2014; Muema et al., 2015). The temporal changing trends of the abundance of AOB *amo*A gene after the effluent treatments were similar (Fig. 4.16). The slightly higher AOB *amo*A gene abundance in the effluent treatments compared with the control was probably because of the ammonia-N in the effluents that stimulated the growth of AOB following each application. The effect on AOB *amo*A gene abundance

was generally similar by the three different effluents. This again would indicate that the application of CW and TE treatments would have a similar effect as that of UE on the AOB abundance. The slight delay in AOB growth following the second effluent application might be because of other factors limiting AOB growth, e.g soil moisture content (Fig. 4.17). The AOA abundance followed a similar temporal trend as AOB but there was no significant difference among the different effluent treatments. These results agree with those by Di et al. (2009) that AOA is less sensitive to ammonium additions to soil.

Denitrifiers including *nir*S, *nir*K and *nos*Z (clades I and II) generally followed a similar temporal pattern as those of AOB and AOA (Fig 4.18, 4.19, 4.20 and 4.21). The higher denitrifier copy numbers in the TE treatment following the second application was probably because of the high organic carbon content in the TE, which stimulated denitrifier growth. It is known that organic carbon is important for denitrifer activities (De Catanzaro & Beauchamp, 1985; Gillam et al., 2008; Miller et al., 2012).

The trend of general agrobacteria gene copy numbers was similar among all four treatments (Fig. 4.22), and there was no significant difference in general agrobacteria abundance among the different treatments ($P>0.05$). This demonstrated that the application of the effluent treatments had no effects on general agrobacteria. The higher fungi abundance in the TE treatment was probably also related to the higher organic matter and other nutrient contents of the TE (Qin et al., 2015).

4.4.3 Effect of different effluents on plant nutrient concentration and yield

The different nutrient contents of each effluent treatment resulted in some differences in nutrient uptake by the pasture. Therefore, the N and P uptakes and total dry matter of the pasture in the TE and UE treatments were significantly higher than those in the control and CW because of the higher N and P contents in the TE and UE (Fig. 4.24b, Fig 4.25b, Fig. 4.43b and Table 3.1). Similarly, the application of the effluents resulted in higher K, S and Fe uptake than that in the control because of the nutrients contained in these effluents (Fig. 4.26b, Fig. 4.27b and Fig. 4.31b). The higher concentrations of S and Fe in the TE and CW also resulted in higher concentrations of these elements in pasture grown on the CW and TE treatment plots (Fig. 4.27a and Fig. 4. 31a). Those demonstrated that the application of effluent treatments would improve the plant nutrition and uptake of N, P, K and S.

Compared with the control, there was no significant difference in Ca, Mg, Al, Mo and Zn after the application of the effluent treatments. Although the Na, B, Cu and Mn concentrations and uptakes in the control were significantly higher than those in the effluent treatments, there was no significant difference between the application of TE and UE in the uptake of these elements. Importantly, the

application of the TE (or CW) effluent did not result in an increase in heavy metal (As, Cd, Cr, Ni and Pb) in the pasture compared with the UE.

4.5 Conclusions

The application of treated effluent resulted in higher organic matter, total C, total N and Olsen P contents in the soil than the application of the untreated effluent. This indicates that the application of treated effluent produced by the ClearTech® process can improve soil fertility when applied to the soil. The **hypothesis** that "the treated farm dairy effluent would have similar effects on soil fertility indices as untreated standard farm dairy effluent" was therefore rejected. In this research, the application of treated effluent had a better effect on soil fertility than that of untreated effluent.

There was no significant difference in the abundance of nitrifying bacteria, general agrobacteria and fungi after the application of TE compared with that of UE. However, the high organic carbon content in the TE increased the growth and activities of denitrifiers. The **hypothesis** that "the treated farm dairy effluent would have similar effects on soil microbial population growth as untreated standard farm dairy effluent" was also rejected. The application of treated effluent would increase the abundance of denitrifiers.

Land application of clarified water, treated effluent and untreated standard farm dairy effluent generally did not result in major differences in plant growth or plant chemical composition that would be of concern. This would verify the **hypothesis** that "the treated farm dairy effluent would have similar effects on plant yield and plant chemical composition as untreated standard farm dairy effluent".

Chapter 5

General conclusions and recommendations for future research

5.1 General conclusions

In New Zealand, the expansion of the dairy farm industry has resulted in nearly 5 million dairy cattle and the generation of a large amount of farm dairy effluent (FDE) (DairyNZ, 2018). Land application of FDE is considered as an effective method to improve soil fertility and pasture growth because FDE contains water and nutrients. ClearTech® is a new FDE treatment technology to recycle water, improve the utilization of nutrients in FDE, and reduce the contamination of surface water. Therefore, it is important to determine if there are different effects of the application of the effluent treated by ClearTech® on soil and pasture compared with untreated effluent (UE). In this study, the effect of clarified water (CW) and treated effluent (TE) were compared with UE, in terms of their effects on soil fertility, functional gene abundance, plant yield and plant chemical composition, following land application of the different effluents in a field plot study.

5.1.1 Effect of different effluents on soil fertility

Results showed that the application of TE resulted in higher contents of soil organic matter, total C, total N and Olsen-P compared the application of UE. The use of PFS also resulted in significantly higher contents of S in the TE and CW treatments than in the UE. There was no significant difference in the contents of NH_4^+-N, NO_3^--N, and CEC in the soil after the application of TE compared with the application of UE. This demonstrates that the application of TE and CW produced by the ClearTech® would improve the soil fertility just as well as, or better than, the application of UE. Therefore, the **hypothesis** that "the treated farm dairy effluent would have similar effects on soil fertility indices as untreated standard farm dairy effluent" was rejected. The application of TE in fact had enhanced positive effects on soil fertility above those from the application of UE.

5.1.2 Effects of different effluents on functional gene abundance

The application of TE resulted in a similar pattern of the abundance of AOB *amo*A gene, AOA *amo*A gene, general agrobacteria and fungi as the application of UE. This demonstrated that land application of TE created by the ClearTech® system does not adversely influence the abundance of AOB *amo*A gene, AOA *amo*A gene, general agrobacteria and fungi compared with UE. However, the higher C content in the TE directly led to higher abundance of denitrifiers (including *nir*S, *nir*K and *nos* Z (clades I and II)) than that in the UE treatment. This indicated that the application of TE could increase the population growth and activity of denitrifiers. Thus, the **hypothesis** that "the treated

farm dairy effluent would have similar effects on soil microbial population growth as untreated standard farm dairy effluent" was partly comfired and partly rejected. The use of ClearTech® did not result in different population abundance of ammonia oxidisers compared with the application of UE, but stimulated the growth of dentirifiers above those by the application of UE.

5.1.3 Effects of different effluents on plant nutrient concentration and yield

Results showed that the pasture N, P, K, Ca, Mg, Al, Mo and Zn uptakes and total dry matter yields after the application of TE were not significantly different from those in the UE treatment. The application of the effluents did not increase the uptake of heavy metal (including As, Cd, Cr, Ni and Pb) in the pasture compared with the control. This indicated that the ClearTech® treatment would not change the pasture nutrient uptakes compared with the untreated effluents. However, the use of PFS increased the S and Fe uptakes of the pasture after the application of TE and CW compared with that of UE. Therefore, the **hypothesis** that "the treated farm dairy effluent would have similar effects on plant yield and plant chemical composition as untreated standard farm dairy effluent" was mostly verified. The application of TE produced by the ClearTech® treatment did not result in major different plant growth and plant chemical composition and only increased the uptake of S and Fe in the pasture compared with the UE.

In conclusion, land application of ClearTech® effluent (TE) would improve the soil fertility, increase the abundance of denitrifiers and not change the abundance of AOB, AOA, general agrobacteria and fungi, plant growth, and plant chemical composition compared with the untreated effluent.

5.2 Future research

The research reported in this thesis was a short-term study and the effluents were applied only twice during the experiment. The influence of different climatic conditions between years on these results are not clear. Long-term studies are justified to determine the effects of the application of the different effluents.

The effluents were applied in the spring and summer in this study. The effect of the applications in other seasons on soil fertility and plant growth would also warrant further studies.

The effect of the application of the different effluents on greenhouse gas emissions (espically nitrous oxide) would also be worthy of investigation.

Finally, there is also a need to study the long-term effects of the different effluents on soil properties and plant growth in other soil types.

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