

# The Effects of Salinization and De-Salinization on Soil Nutrients and Organic Matter in Tidal Freshwater Wetlands

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Master Thesis



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Date: **23-07-2012**



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Master Thesis Sustainable Development, track Global Change & Ecosystems

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## Foreword

This research was conducted as a final assignment for the master program Sustainable Development at the Utrecht University. It has been a quite an experience for me to spend three quarters of a curriculum on a single research topic and to think and work through an entire research from the concept to the actual end product. All in all I've learnt quite a lot in regard to the scientific process, but also how I can deal with unforeseen situations flexibly. I would like to use this opportunity to thank my supervisor, Aat Barendregt, for his input during our brainstorm session and the critical view on my work. Dineke van de Meent has helped me with the laboratory work and sample analysis for which I am most grateful. I'd like to thank the 'Zuidhollands Landschap' foundation for granting me permission to access the tidal wetlands. And last but not least, I would not have been able to get this far without the unlimited support of my parents. Especially the enthusiasm and practical approach to the fieldwork from my father is much appreciated.

Peter

## **Abstract**

Future sea level rise and changes to river discharge due to climate change will periodically alter the tidal freshwater wetland water quality. This will lead to exposure of tidal freshwater wetlands to alternating saline and fresher conditions. The effects of increased and decreased salinity on fresh and saline wetland soils nutrients and organic matter has been measured using a manipulative field-experiment displacing soil samples from saline tidal wetlands to tidal freshwater wetlands and vice versa. Pore water analysis and loss-on-ignition tests of the extracted soil cores have been used to observe changes in nitrate, ammonium, phosphate and organic matter. The soil LOI-analysis did not show a significant change of organic matter when exposed to increased or decreased salinity on a natural site. The fresh conditions on a restored site lowered the amount of organic matter in saline soils. Increased salinity decreases the pore water phosphate and nitrate concentration and increases the soil nitrate concentration. For decreased salinity the soil conditions seem an important factor: the restored wetland conditions reduce phosphates in the pore water, whilst they increase in the natural wetland. On both sites reduced salinity leads to an increase in the ammonium concentration and a reduction in the nitrate concentration. More research is required to pinpoint the main active biological and chemical processes.

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## Introduction

Tidal freshwater wetlands (further also referred to as TFW) are naturally occurring dynamic ecosystems with a specific set of physical requirements. They only occur in the intertidal zone and are thus exposed to regular flooding and require oligohaline or freshwater conditions (Whigham, 2009; Barendregt et al. 2006). TFW are mainly found in upstream estuarine areas where tidal influences ensure diurnal flooding but where the water salinity can be considered low. These hydrologic requirements minimize salt stress on vegetation and micro-organisms which add to the biological and ecological value of TFW (Weston et al. 2010). On a broader perspective tidal freshwater wetlands can be seen as part of the larger estuarine ecosystem. Within the estuarine ecosystem TFW play an important role in providing habitats for birds and provide spawning grounds for multiple fish species. This indicates that TFW can be an asset to estuarine ecosystems by increasing the natural value, increasing diversity and providing niche habitats for specific species (Struyf et al. 2005; Struyf et al. 2006).

Besides the apparent ecological value of tidal freshwater wetlands they also fulfill a range of functions valuable to humankind (Costanza & D'Arge, 1997). Meire et al. (2005) for example show that TFW can aid the mitigation of flooding and characterize these biomes as follows: Tidal Freshwater Wetlands *"are very productive biomes and support many important ecosystem functions: biogeochemical cycling and movement of nutrients, mitigation of floods, maintenance of biodiversity and biological production"*. Seitzinger (1994) indicates that TFW are known sinks for natural and anthropogenic riverine nitrogen inputs. Chemical processes in tidal freshwater wetlands play an important role in mitigating contaminant transport in the estuary, thereby enhancing the estuarine water quality (Noegrohati et al. 2008). The latter is of significance since estuarine freshwater bodies are important sources for agricultural- and drinking water (Costanza & D'Arge, 1997). Sharp et al. (1984) address some of the active processes: *"Adsorption, flocculation, biochemical processes and precipitation [in tidal freshwater wetlands] aid pollutant removal"*. In short TFW ecosystems can be seen as filters acting against riverine pollutants such as nutrients. They help reduce exposure of estuaries and coastal areas to these substances while also helping to maintain an important source of agricultural and drinking water clean. An increased nutrient load in estuarine water due to dysfunctional TFW filtering mechanisms could lead to algae bloom which damages downstream estuarine and marine ecosystems.

## Active Processes

The previously mentioned hydrologic requirements (diurnal flooding and low salinity) ensure a high soil moisture content. It also minimizes salt stress on vegetation and micro-organisms which are of ecological significance but are also important to the TFW-filtering function (Weston et al. 2010). These requirements also allow specific chemical reactions in the soil to occur (Mahrous et al. 1983; Neubauer, 2011).

Neubauer (2011) has shown that TFW have been able to exist in symbiosis with a natural change of sea level for millennia due to a high sedimentation rate, gradually elevating the soil level in tandem with sea level rise. Climate research however indicates that in the future TFW water availability may be altered in two ways: First global climate change enhances the sea level rise compared to the naturally occurring sea level rise (Church & White, 2006; IPCC, 1992). Secondly climate change may also alter the river discharge regime (Pezeshki et al. 1990; Doyle et al. 2007; Kraus et al. 2009; Jun & Craft, 2012 to be published).

It is unknown how TFW will respond to a faster sea level rise and if they for instance are able to keep pace. The current prognosis for the Netherlands is that the riverine discharge will decrease during summer and increase during wintertime due to changing precipitation and evaporation patterns and increased snowmelt in the river catchments (NOP, 1999).

The expected influence of an increase of the sea-level on TFW are a longer inundation time and more TFW areas being inundated, but also alteration in the estuarine water quality. An increased sea level could result in an upstream movement of the interface between riverine freshwater and saline seawater, known as the salt wedge (Savenije, 2005). This could result in an increased salinity of estuarine water near a TFW (Kurup, Hamilton & Patterson, 1998). A changing river discharge regime may enhance these effects on a TFW water conditions in two ways (Kurup, Hamilton & Patterson, 1998): A limited freshwater supply in an estuary during seasonal low river discharge will increase the influence of the sea on the estuarine water. This would allow the salt wedge to intrude further into the estuary increasing the salinity further upstream, where TFWs are situated. During seasonal periods of high discharge the increased river water supply may be able to force the salt wedge downstream and thereby reduce the salinity of water entering the TFW. If the increased sea level rise continues and expected river discharge patterns occur it is thus likely that the Dutch TFW will be exposed to alternating salinization and desalinization.

### Known effects

A lot of research is conducted to investigate the effects of increased water salinity on soil chemistry, microbial activity, flora and fauna. Increased salinity in TFW ecosystems is known to alter vegetation (Baldwin, 2011; Kraus et al. 2007; Bernstein, 1975) and occurring aquatic and soil microbial life (Peelen, undated). It also alters biochemical carbon mineralization pathways (Weston et al. 2006), increases organic carbon decomposition (Craft, 2007; Craft et al. 2008) reduces nitrogen retention and increases phosphorus sorption (Jun & Craft, 2012 to be published). Exposure of anaerobic freshwater soils, found in the intertidal zone of TFWs, to high levels of salt alter organic carbon remineralization processes from methanogenesis to sea-salt supplied sulfate-reduction (Capone & Kiene, 1988) and increases the carbon decomposition speed (Weston et al. 2006). The increased carbon mineralization reduces the soil volume and thereby undermines the previously mentioned TFW high dependency on (organic) sedimentation to remain above sea level (Neubauer, 2011). Salt intrusion could thus lead to a loss of tidal freshwater wetlands.

The nitrogen retention function of TFW is based on multiple biological and chemical systems: Nitrogen occurs in many forms ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_3$ ,  $\text{NH}_4^+$ ) and can be bound to organic matter and soil particles. Nitrogen can be released and made bio-available via mineralization of organic matter by micro-organisms (Craft, 2007; Weston et al. 2006; Jun & Craft, 2012 to be published), but also be released from soil particles due to an increased ionic-strength due to an increased salinity (Weston et al. 2010). The bio-available  $\text{NO}_3^-$  can be taken up by plants and stored in their tissue, released to the atmosphere in an anaerobic denitrification process by soil bacteria or transformed to ammonium ( $\text{NH}_4^+$ ) in an anaerobic ammonification process. If seasonal alteration between fresh and salt conditions increases mineralization of organic matter soil  $\text{NO}_3^-$  is expected to reduce, and  $\text{NH}_4^+$  is likely to change depending on the salt tolerance of ammonifying bacteria. It is to be expected that seasonal exposure to lower salinity releases the cations from the salt water allowing un-leeched  $\text{NO}_3^-$  or  $\text{NH}_4^+$  ions to re-sorb to soil particles.

Increased Phosphorus sorption under increased salinization is thought to be induced by adsorption/desorption mechanisms and ion exchange (Reddy et al. 1999). Darke & Walbridge (2000) however suggest that flooding induced Iron and Aluminum reduction are more important factors in phosphorus sorption. Their research however does not indicate a change in phosphorus sorption from temporal salinization or possible recovery due to temporal desalinization. Since the soil in TFW's is often reduced due to regular flooding it is likely that the pore water phosphorus content is low. Salinity-induced organic matter mineralization would release phosphorus bound in organic matter allowing it to leech or bind to soil particles.

### **Focal points & research questions**

In contrary to the effects on freshwater soils exposed to salinization very little research is done on the effects of the expected desalinization in TFW soils. This information however could help determine the vulnerability of TFW to expected seasonally alternating salt conditions and could provide a basis for protective measures. It is therefore important to assess the capability of tidal freshwater wetland soils to recover from temporal salinization. There is no data available on the recovery of the soil nitrogen retention-function, the phosphorus sorption-function or carbon mineralization processes of freshwater soils exposed to alternated salinization and desalinization.

The focus of this research lies on the ecosystem functions concerning biogeochemical nutrient cycling in tidal freshwater wetland areas and aims to determine the effects of increased salinization and desalinization due to sea level rise and a changing river discharge pattern on soils in TFWs in order to increase knowledge of TFW's resilience to changing water conditions. The main research question is therefore:

*What are the effects of salinization and desalinization on tidal freshwater wetland available soil nutrients and organic content?*

To answer this research question the following four sub questions are to be answered:

- 1) *What is the effect of salinization on the availability of nutrients in tidal freshwater wetland soils?*
- 2) *What is the effect of salinization on the amount of organic matter in tidal freshwater wetland soils?*
- 3) *What is the effect of desalinization on the availability of nutrients in salinized tidal freshwater wetland soils?*
- 4) *What is the effect of desalinization on the amount of organic matter in salinized tidal freshwater wetland soils?*

Based on the literature described in the above chapter I hypothesize that an increased salinity will increase organic carbon mineralization (decrease carbon) and increase  $\text{NO}_3^-$  release. Salinity will also remove phosphate from the pore water. Little is known on the effects of desalinization in TFWs, however based on the fact that TFW's are highly resilient ecosystems I expect that a reduction of salinity will allow the ecosystem to recover, slowing the carbon mineralization process down, increase nitrogen retention and release of sorbed phosphates. The duration of this research however will likely be insufficient to indicate a possible regeneration of soil organic matter.



## Methods

As stated in the previous chapter, research by Weston et al. (2006) but also by Megonigal & Neubauer (2009), indicates that salt stress on freshwater ecosystems may alter the biogeochemical properties in the TFW-soil. These chemical processes are an important asset to the ecological functions of estuarine wetland areas, meaning that increased salt stress will lead to the diminishing of some of the functions a TFW provides. An increased mineralization of organic matter may lower the ground level which could disturb the balance between sea level rise and soil level rise (Neubauer, 2011). There is however little known on the effect of *desalinization* of TFW soils exposed to salinization.

The process of increased seasonal salinization and desalinization has not yet set in. Therefore it is impossible to do real time field observations. Instead was opted for a manipulative field experiment focused on exposing TFW soil samples to higher salinity and more saline wetland soils to lower salinity. Due the limited timeframe the seasonal alteration between water conditions was neglected.

## Research Sites

The sites chosen for this research are three nearby tidal wetlands in the Dutch province of Zuid-Holland maintained by the 'Zuidhollands Landschap' foundation. Each site is situated near a river and exposed to diurnal flooding. The sites have been selected based on their current and historic use and most importantly the electric conductivity of the water entering the system during high tide. Electric conductivity was measured using an EC-meter on 4 pre-experimental site visits during high and low tide and is used as an indicator for water salinity confirming observations by Kers et al. (2001). On each location a site was chosen for the experiments with specific attention to the elevation compared to the high-tide mark to ensure equal flooding characteristics. Attention was also paid to soil disturbances such as roots which can influence oxygen and nutrient conditions.

Site A is the 'Ruigeplaatbos' near the village of 'Hoogvliet'. This site is located most seaward of the three wetlands and is situated near the salt wedge (Kers et al. 2001). The river water has an electric conductivity (EC) of approximately 3000 to 4000  $\mu\text{S cm}^{-1}$  during high tide, meaning that the water salinity ranges from brackish to salty. During low tide with high river discharge however the EC is lowered to 700-800  $\mu\text{S cm}^{-1}$ , comparable to river water. This river water however does not often enter the wetland due to the tide being low. The 'rijksdriehoek' coordinates to the sample site are: RD082.638-431.511.

Site B is the 'Klein Profijt' situated on the north-bank of the 'Oude Maas' near the village of 'Heinenoord'. It has dominant freshwater condition with an EC of approximately 700-800  $\mu\text{S cm}^{-1}$ . The observation location here slightly differ from the other two locations for the main reason of accessibility: The tidal sand/mudflats are rough and inaccessible, thus the experiments were set closer to shore. It is also important to note that this site has been restored to a more natural state about a decade ago after being used as a harbor sludge deposit. Due to the latter the phosphate and ammonium content are higher than the other two sites while the organic content and nitrate are considerably lower. Including this location could prove to be a valuable addition in regard to contrast in the observations. The coordinates to the exact sample site are: RD090.017-427.874.

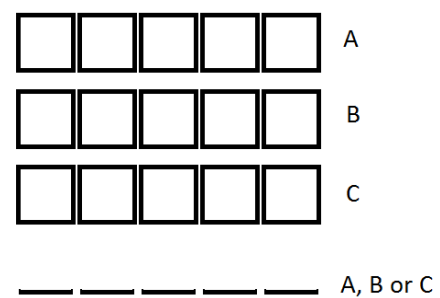
Site C is the ‘Stormpoldervloedbos’ near the village of ‘Krimpen-aan-den-IJssel’ and is situated most land-inward from the three sites along the ‘Nieuwe Maas’ river. The EC is similar to site B and approximates  $650-800 \mu\text{S cm}^{-1}$ . The coordinates to this sample site are: RD099.815-435.464.

## Experimental Design

The experiment is designed to expose fresh soil to an elevated salinity and salinized soil to fresh conditions to mimic the effects of seasonal salinization and de-salinization. The samples have been set out on March 19<sup>th</sup> and are exposed to the natural conditions of the specific sites until May 8<sup>th</sup> 2012. The first two weeks of the experiment temperatures occasionally dipped below freezing, they could have reduced biological and chemical processes.

From each research site a total of 10 random soil samples have been taken which are divided over the other two sites. These exchanged samples are held in 1000mL plastic containers which are filled to the rim and are slightly compressed in order to restore the soil structure. Specific attention was paid to the sampled soil in regard to absence of disturbances (large roots, germinating plants, rocks, et cetera). Relocating the samples was done within the same tidal stroke to ensure an equal exposure to tidal influences and thus allow for an equal comparison. The soil samples are placed on the new locations inside the plastic container to ensure identification of the samples after exposure. The containers are marked and pierced multiple times to allow soil water to infiltrate the sample and allow for possible lateral flows. During relocation all samples were placed level to the surrounding soil and fit in a gap with as little clearance as possible to prevent water from infiltrating excessively along the sides. From each site a schematic drawing was made to identify each sample after exposure. Besides the exchanged soil samples the same procedure is executed without the exposure. On each location 5 additional soil samples are taken and placed into similar containers. These however are not exchanged but placed back into the soil to assess the possible influence of the experimentally altered conditions on the samples. All soil samples have been exposed to the experimental conditions for 6 weeks. In total each site contained 15 experimental soil samples (10 containing soil from the other two locations and 5 with soil from the own location).

From each 1000mL container two random soil cores were taken for analysis using a soil-core sampler. After sampling the cores were stored in 50mL marked and capped plastic tubes and cooled to reduce nitrogen transpiration and carbon decomposition during transport. Besides the experimental soil cores an additional 10 cores are taken from the full soil without the experimental conditions for verification of possible influence of the experiments. In total each site yields 10 experimental soil cores from location A, 10 experimental cores from location B, 10 experimental cores from location C, plus an additional 10 from the full soil. The following tables clarify the relation of the number of experiments to the number of samples for different analysis. The relation between the number of experiments and number of samples for analysis is displayed in table 1-4 on the next page:



**Figure 1. Schematic overview of the experimental setup on one location. Each cube represents a 1000mL soil container. Horizontal lines represent non-experimental full-soil samples. Letters indicate the soil source, these vary for the full soil samples depending on the location. From each experimental and non-experimental container 2 samples are taken.**

Table 1 below indicates the number of experiments per location and the source of the experimental soil.

Source\Destination	Location A	Location B	Location C
Location A	5*	5	5
Location B	5	5*	5
Location C	5	5	5*

Table 1. Number of experiments per location and the source of the experimental soil. \*=non-exchanged experiments, used for the experimental verification with the non-experimental samples

From each experiment 2 soil samples are taken for pore water extraction. The total number of soil samples is indicated in table 2 below:

Source\Destination	Location A	Location B	Location C
Location A	10*	10	10
Location B	10	10*	10
Location C	10	10	10*
Non –experimental	10	10	10

Table 2. Number of samples per location and source. \*=non-exchanged experiments, used for the experimental verification with the non-experimental soil samples.

The pore water samples are used for two different laboratory analyses (ICP: NO<sub>3</sub> & Dynex Auto-analyser: NH<sub>4</sub>, PO<sub>4</sub>), the total number of Nitrate, Ammonium and Phosphate samples are therefore half the number of pore water samples as indicated in table 3:

Source\Destination	Location A	Location B	Location C
Location A	5*	5	5
Location B	5	5*	5
Location C	5	5	5*
Non –experimental	5	5	5

Table 3. Number of pore water samples for ICP or Dynex analysis per location and source. \*=non-exchanged experiments, used for the experimental verification with the non-experimental soil samples.

For organic matter each soil sample was used for two LOI-analysis. The total number of organic matter observations thus is twice the number of soil samples that have been indicated in table 2:

Source\Destination	Location A	Location B	Location C
Location A	20*	20	20
Location B	20	20*	20
Location C	20	20	20*
Non –experimental	20	20	20

Table 4. Number of samples for LOI-organic matter observations per location and source. \*=non-exchanged experiments, used for the experimental verification with the non-experimental soil samples.

## Laboratory Analysis

Each soil core was centrifuged to release the pore water. This water was extracted using a syringe and filtered using a 45µM filter to remove particulates and prepare it for analysis. The filtered pore water samples are stored in 10mL capped flasks. Five pore water samples are analyzed using a Dynex auto-analyzer ( $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ) and the other five using ICP-OES ( $\text{NO}_3^-$ ). To summarize: per location 20 pore water samples have been analyzed using Dynex, and 20 using ICP-OES.

The remaining centrifuged soil samples were subsequently mixed to a homogenous moist 'paste' and dried in a stove at 70°C for 24 hours to remove all remaining moisture. The average organic content (AOC) of the dried soil samples was determined using the LOI-method (Loss On Ignition) described by Servais, Anzil & Ventresque (1989) and Nelson & Summers (1996). The dry samples are weighed (DW) and incinerated in an autoclave at 450°C for 5 hours ( $W_{450}$ ). The LOI (%) was calculated as follows:

$$\text{LOI (\%)} = ((\text{DW} - W_{450})/\text{DW}) * 100\%.$$

The specific incinerator temperature and time have been selected to prevent the incineration of volatile salts and carbonates such as  $\text{CaCO}_3$  or  $\text{MgCO}_3$  (Heiri et al. 2001) which could influence the LOI-observations, but on the other hand maximize oxidation of organic matter.

## Analysis, statistics & constraints

Since the number of tidal wetlands is limited due to the Dutch spatial planning and intervening in the river systems the choice for experimental locations was limited to what was available. Location A can be considered a natural system with saline influence, location C a natural system without saline influence, and location B a restored natural system without saline influence. Especially the restoration of location B can prove to be an important factor in this analysis: this location likely did not have had time to build ample amounts of organic matter and leaching of nutrients from the recently removed sludge storage may have increased these nutrient contaminants in the soil. The best comparison of effects will however be the soil from location A exposed to the conditions on location C and vice versa. The different nutrient concentrations on location B however can be seen as an exaggerated comparison and may prove useful in pinpointing effects otherwise obscured by measurement errors or lost in the analysis sensitivity range. It is important to note that observations from location B are more to be seen as an indicator.

In order to compare the different groups of samples a statistical analysis is required. The following tests have been done in order:

- 1) Verify the data groups for outliers using SPSS
- 2) Remove the outliers and test data groups for being a 'normal distribution'
- 3) Applied independent sample t-tests between groups for comparison using Excel
- 4) Averages and standard deviations are calculated

## Results

This chapter contains the experimental result, which are presented in bar graphs including an error-bar containing the respective standard deviations. Each graph is accompanied by a table containing the relevant data for the number of samples, the average per group and the standard deviation. The results are divided in four sub-chapters in order to differentiate between the soil Average Organic Content (AOC), the pore water Average Phosphorus Content (APC), Average Ammonium Content (AAC) and the Average Nitrate Content (ANC) measurements. Finally a brief summary of the results will be presented in order to integrate the observations.

### Outliers

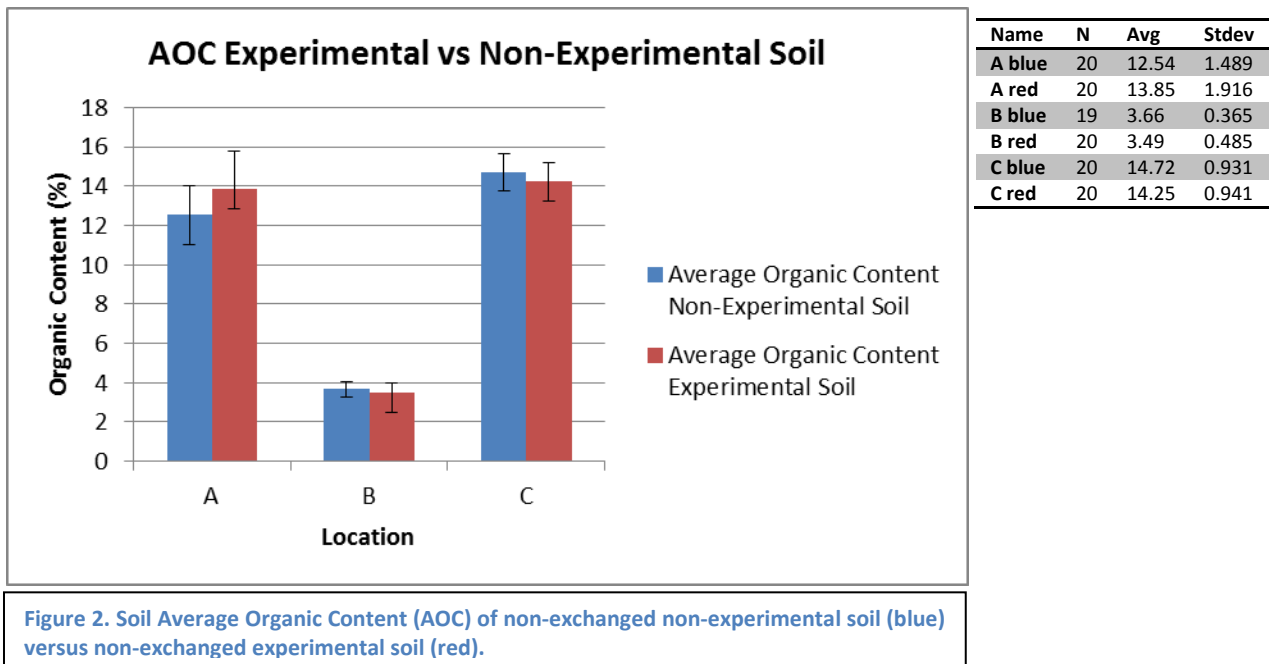
All soil-LOI, pore water auto-analyzer and ICP-data was checked for outliers using SPSS for each group of samples. In total four were found in the LOI-measurements. Striking is that these figures were all on the higher side. The most likely explanation is the possibility of unnoticed organic macro particles being in the incinerated samples. The outliers have been removed from the data. There were no outliers found in the Dynex and ICP data, likely due to the limited number of samples.

## Organic Content

The average organic content (AOC) of the soil was analyzed using the previously described LOI-method and is expressed as a percentage of the dry sample soil weight. Per location 80 LOI-measurements have been made, twenty for each type of experiment. Four outliers have been identified and removed from the observations.

### AOC Experimental verification

In order to determine the effects of the experimental conditions on the average organic content of the soil the non-experimental non-exchanged soil (blue) was compared to the experimental non-exchanged soil (red). The results are presented below in figure (2):



As the figure 2 indicates no significant difference between the experimental and non-experimental results can be observed on site A ( $A \alpha=0.0687$ ), site B ( $B \alpha=0.9549$ ) and site C ( $C \alpha=0.0837$ ). Based on these results the conclusion can be drawn that the experiment did not significantly alter the organic content on either of the sites: the non-experimental and experimental soil can be seen as equal. This result is taken into account for the analysis to come, the non-exchanged experimental and non-experimental AOC samples have been merged to a single larger group in order to increase the number of samples and strengthen the value of the statistical conclusions.

### AOC Exchange Fresh to Fresh

The next figure (3) shows the soil average organic content of the experimental fresh soil exchanged to the other freshwater-site. The non-exchanged experimental and non-experimental data groups have been combined to enhance the number of samples N.

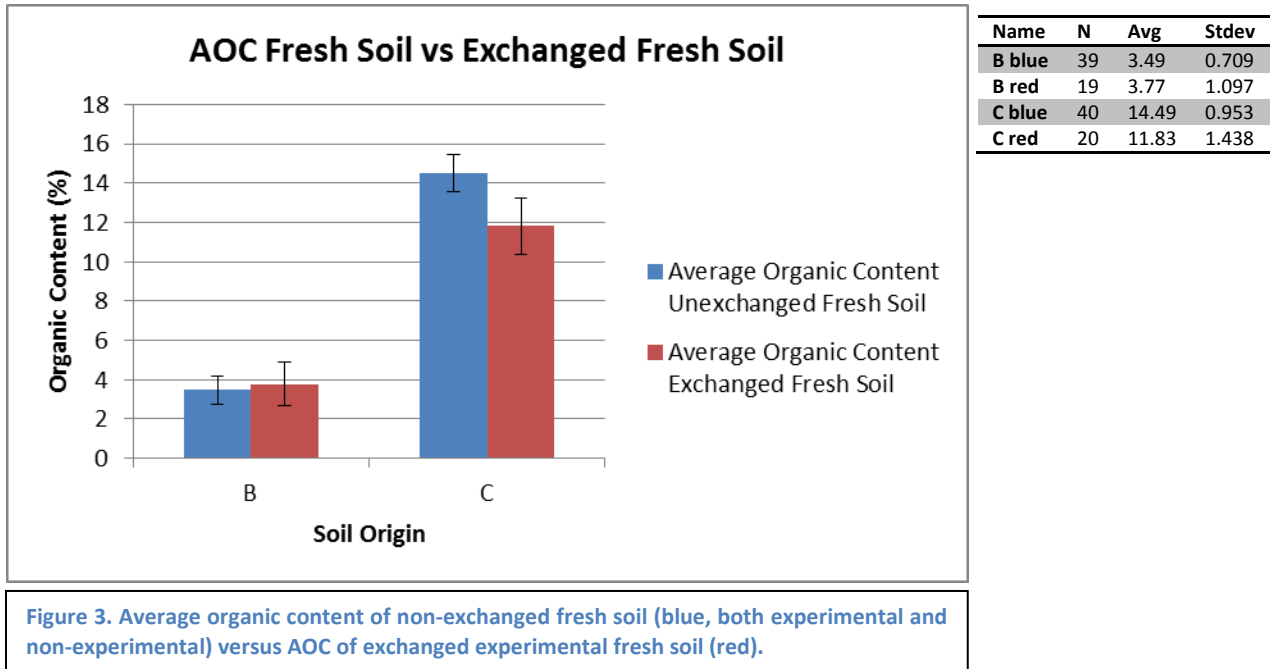
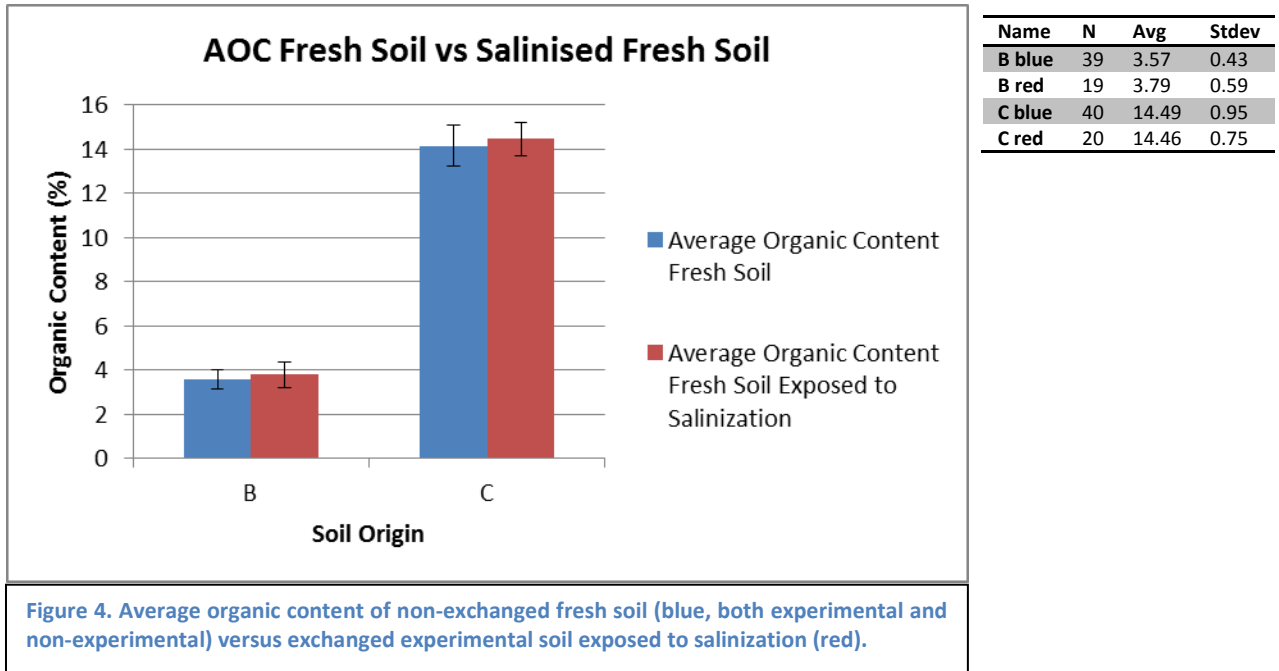


Figure 3. Average organic content of non-exchanged fresh soil (blue, both experimental and non-experimental) versus AOC of exchanged experimental fresh soil (red).

The Fresh soil originating from location B was exposed to the conditions on site C. These samples do not show a significant change in organic matter (B  $\alpha=0.2927$ ). The soil samples from site C, exposed to conditions of site B in contrary do shows a small but significant decrease in organic content (C  $\alpha<0.05$ ) in contrary to the expectation that the conditions are similar.

### AOC Exchange Fresh to Salt

The next figure (4) shows the soil average organic content of fresh soil samples exposed to the higher salinity conditions on site A. Similar to the previous image the non-exchanged experimental and non-experimental samples have been combined to increase the N.



In contrary to the hypothesis both sites show a small increase of organic content of the fresh soil exposed to higher salinity. Statistics however indicate these increases are not significant (B,  $\alpha=0.1633$ ) (C,  $\alpha=0.8959$ ).



### AOC Exchange Salt to Fresh

Figure (5) shows the average organic content of saline soil exposed to the fresher conditions on locations B and C. It is important to note that in contrary to the previous figures all soil originates from one site (A) and has been placed on the other two sites (B and C). Again the non-exchanged non-experimental soil samples have been combined with the non-exchanged experimental samples to increase N.

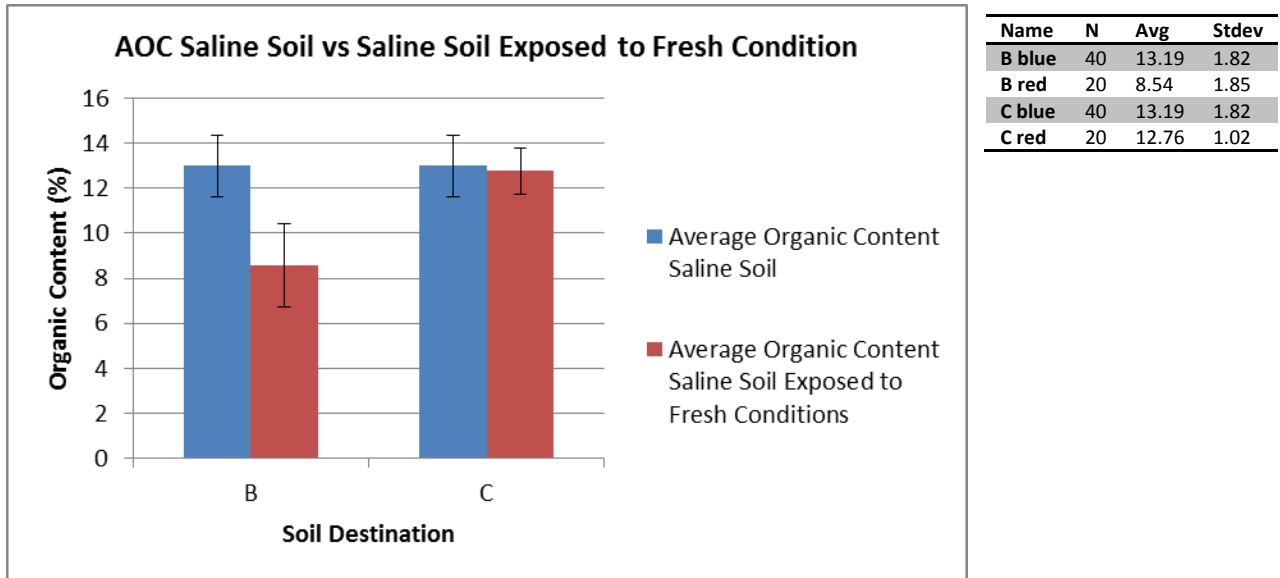


Figure 5. Average organic content of non-exchanged saline soil (blue, both experimental and non-experimental) versus exchanged experimental soil exposed to fresh conditions (red). Note that in contrary to the previous figures all soil originates from location A, the horizontal axis thus represents the location where this soil was placed and not the origin.

The saline soil exposed to the fresh condition on location B shows a significant decrease ( $B \alpha < 0.05$ ), while the same soil exposed to the condition on site C does not show a decrease ( $C \alpha = 0.2362$ ).

## Phosphate

The pore water phosphate ( $\text{PO}_4^{3-}$ ) content was measured using a Dynex auto-analyzer. Due to limited resources the number of pore water average phosphate content (APC) observations per location is smaller than the soil average organic content observations. Therefore the conclusions derived from these observations may be less accurate. Again all data groups have been checked for outliers, yet the small number of observations did not allow clear pinpointing of a possible outlier. In some occasions this has resulted in considerably high standard deviations.

### APC Experimental verification

Similar to the measurements of the soil organic content the pore water phosphate content observations are also checked in order to rule out the possible influence of the experimental conditions on the observations. Figure 6 shows the pore water average phosphate content of the non-experimental non-exchanged soil versus the experimental non-exchanged soil.

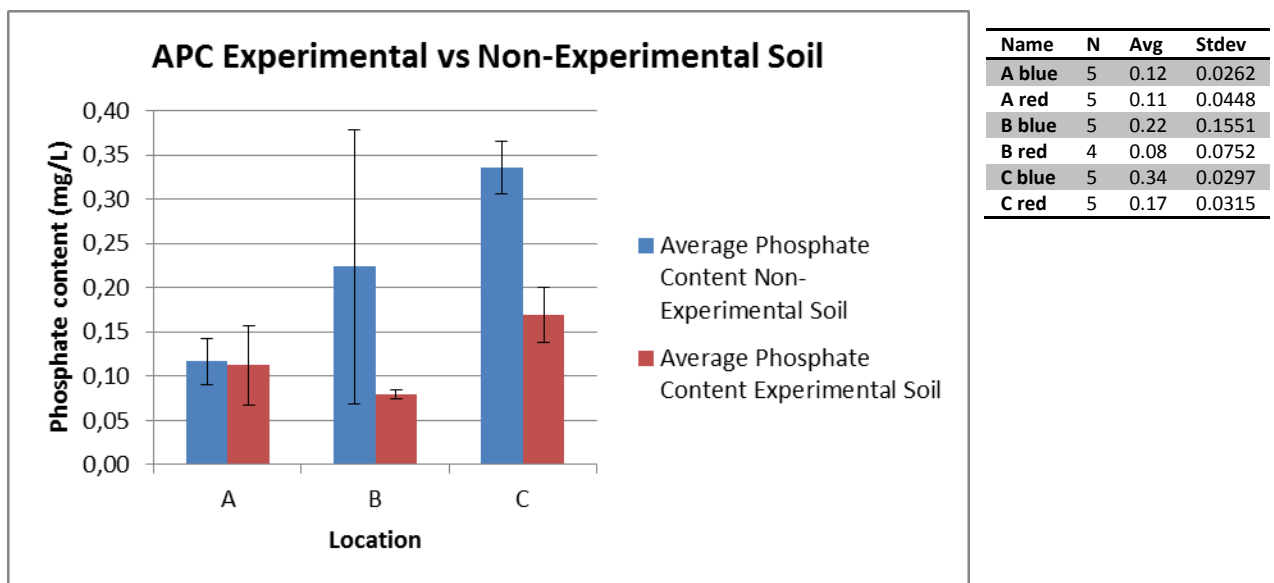
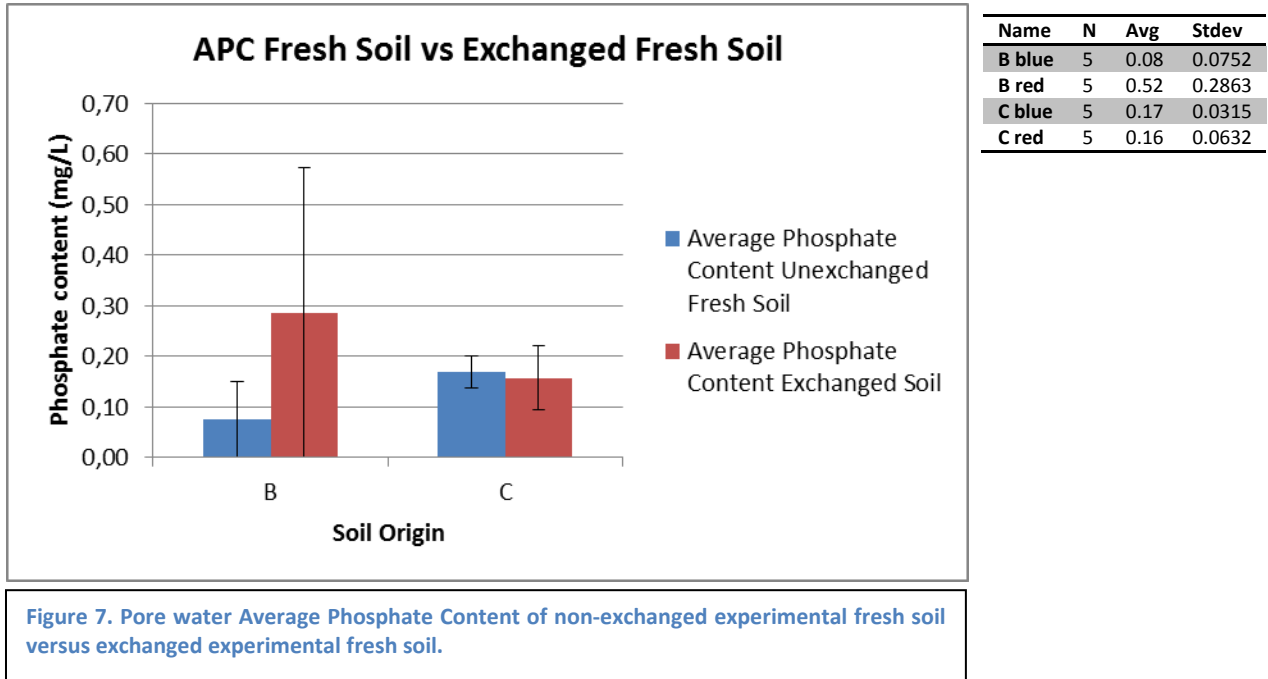


Figure 6. Pore water Average Phosphate Content (APC) of non-exchanged non-experimental soil (blue) versus non-exchanged experimental soil (red). Note: due to low pore-water yields the number of observations on the experimental soil in B red is limited to 4. A possible outlier has resulted in the high average and standard deviation in the non-experimental soil on location B (blue).

There is no change in the pore water phosphate content observable in the samples from location A ( $\alpha=0.867$ ). On location B also no significant difference is observable, however there is a large difference in the standard-deviations between the experimental and non-experimental sites. This is likely due to the fact that an outlier could not be definitively be excluded (0.47mg/L) ( $B \alpha=0.1047$ ). On location C a significant difference was found between the experimental and non-experimental observations ( $C \alpha<0.05$ ).

### APC Exchange Fresh to Fresh

Figure (7) below shows the effect of experimental fresh soil exposed to the conditions on the other fresh location. The previous experimental verification tests did not allow enhancement of the group size by combining the non-exchanged experimental and non-experimental samples leaving the N at 5.



The small dataset has resulted in weak statistical conclusions, especially on site B ( $\alpha=0.0696$ ) due to limited possibility to pinpoint and exclude outliers. The pore water phosphorus content from site C exposed to conditions on site B however does indicate that the conditions on the exchanged site did not alter the soil characteristics significantly ( $C \alpha=0.4667$ ).

### APC Exchange Fresh to Salt

The pore water average phosphate content of soil from site B and C exposed to conditions on site A is displayed in figure (8) below. Since the results on the experimental verification test did not show a similarity the experimental non-exchanged samples are compared to the experimental exchanged samples without the group-enhancement.

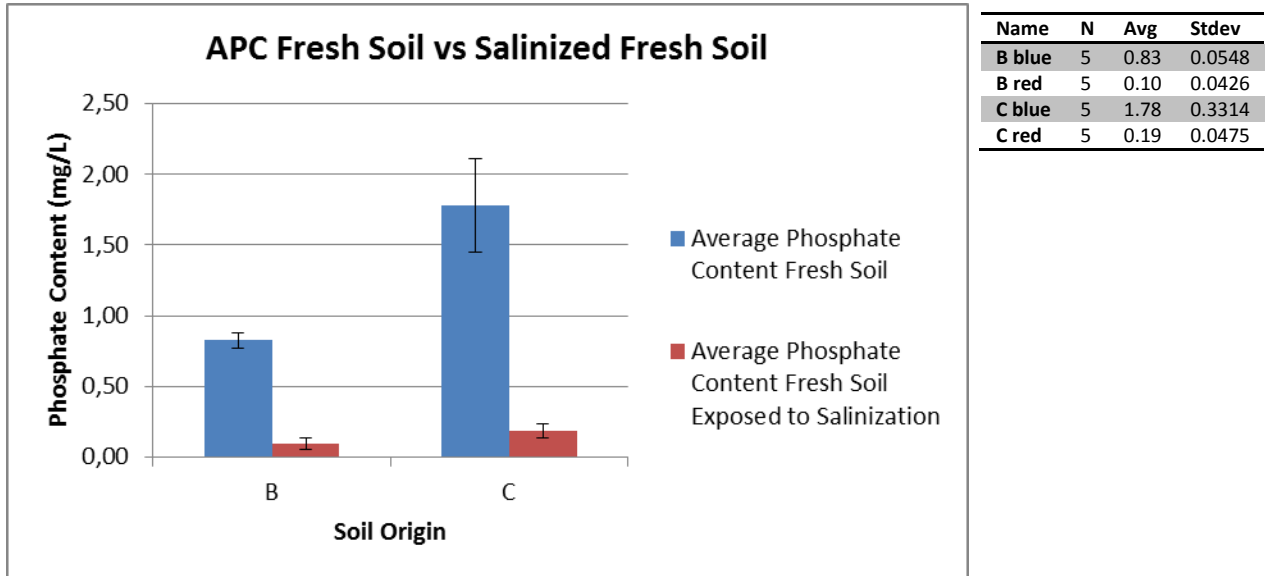


Figure 8. Average phosphate content of fresh soil versus exchanged fresh soil exposed to increased salinity.

Figure (8) indicates that the pore water average phosphate content on site B did significantly reduce due to increased salinization ( $B \alpha < 0.05$ ). The soil on site C also shows a significant decrease in the amount of phosphate in the pore water when exposed to increased salinity ( $C \alpha < 0.05$ ).

### APC Exchange Salt to Fresh

In contrary to the previous experimental comparisons the non-experimental and experimental samples did not significantly differ (see phosphate experimental verification). Therefore in this case the saline experimental and non-experimental samples can be merged together to enhance the group size. Figure (9) shows the effect of reduced salinization on the saline soil pore water phosphorus content.

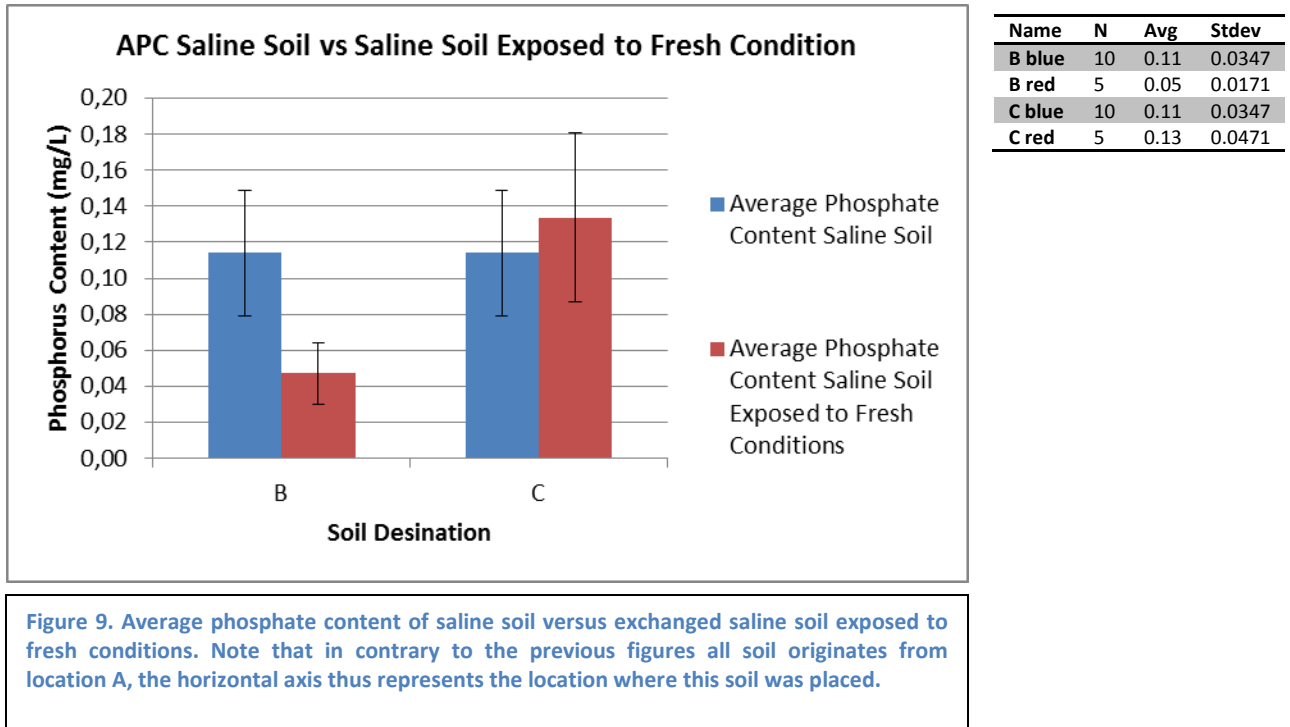


Figure 9. Average phosphate content of saline soil versus exchanged saline soil exposed to fresh conditions. Note that in contrary to the previous figures all soil originates from location A, the horizontal axis thus represents the location where this soil was placed.

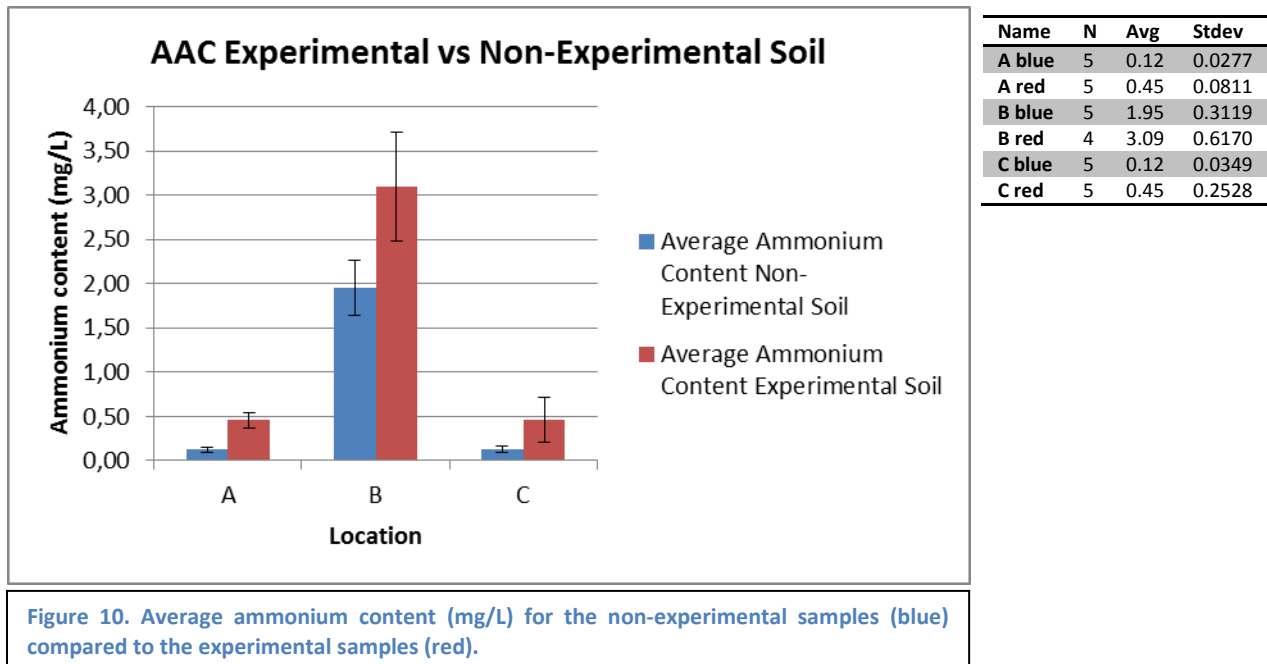
The saline soil exposed to the fresh condition on site B shows a significant decrease in the pore water phosphate content (B  $\alpha=0.000246$ ). The phosphate content of the pore water from saline soil exposed to the conditions on site C does not indicate a significant increase or decrease (C  $\alpha=0.437446$ ).

## Ammonium

The gathered pore water samples that were tested for phosphate have also been tested for ammonium ( $\text{NH}_4^+$ ) during the same Dynex auto-analyzer run. Per site a total of 20 samples have been analyzed, similar to the phosphate samples.

### AAC Experimental Verification

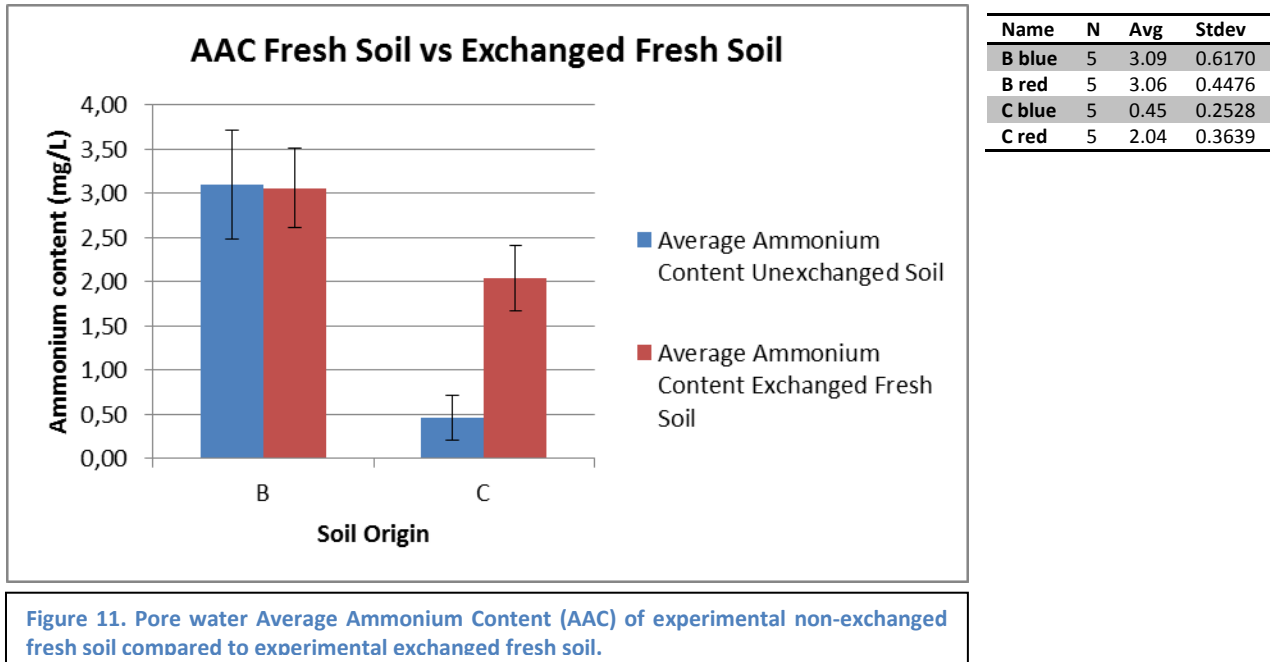
As with the soil AOC and pore water APC the pore water Average Ammonium Content (AAC) was tested to verify the effects of the experimental setup. These are shown in figure (10) below.



On the left figure (10) shows the average ammonium content of the non-exchanged non-experimental samples compared to the experimental non-exchanged samples. In contrary to the expectations the experimental samples are significantly higher (A  $\alpha < 0.05$ ). The AAC for site B is considerably higher, this is explained by the history and recent restoration of this area. There however still is a significant increase between the non-experimental and experimental groups of these samples (B  $\alpha = 0.0256$ ). The AAC level of location C is on a comparable level with observations from location A. The large variation between observations however increased the standard deviation. There however is no significant difference between the non-experimental and non-experimental samples (C  $\alpha = 0.0312$ ).

### AAC Exchange Fresh to Fresh

Figure (11) shows the pore water Average Ammonium Content (AAC) of the experimental fresh soil exchanged to the other freshwater-site. The non-exchanged experimental and non-experimental results have not been combined.



At AAC of the restored soil did not significantly change when exposed to the natural fresh location (B  $\alpha=0.922$ ). The natural soil exposed to the restored location showed a significant increase in the amount of ammonium (C  $\alpha<0.05$ ).

### AAC Exchange Fresh to Salt

The below figure (12) shows the effects of increased salinity on the average ammonium content of fresh exchanged experimental soil.

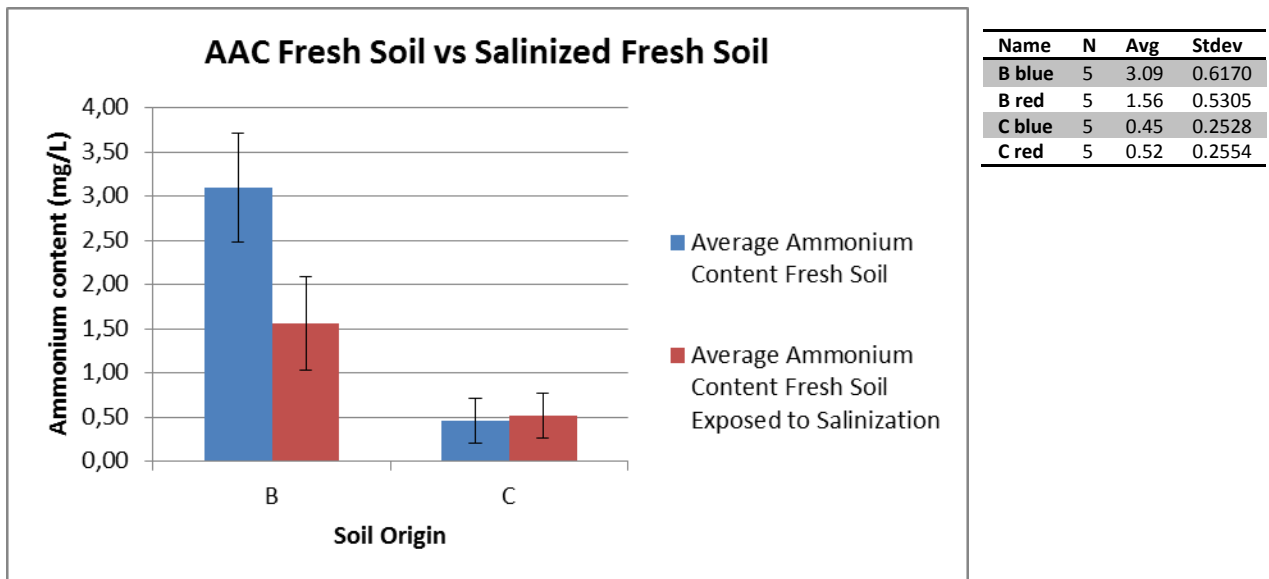


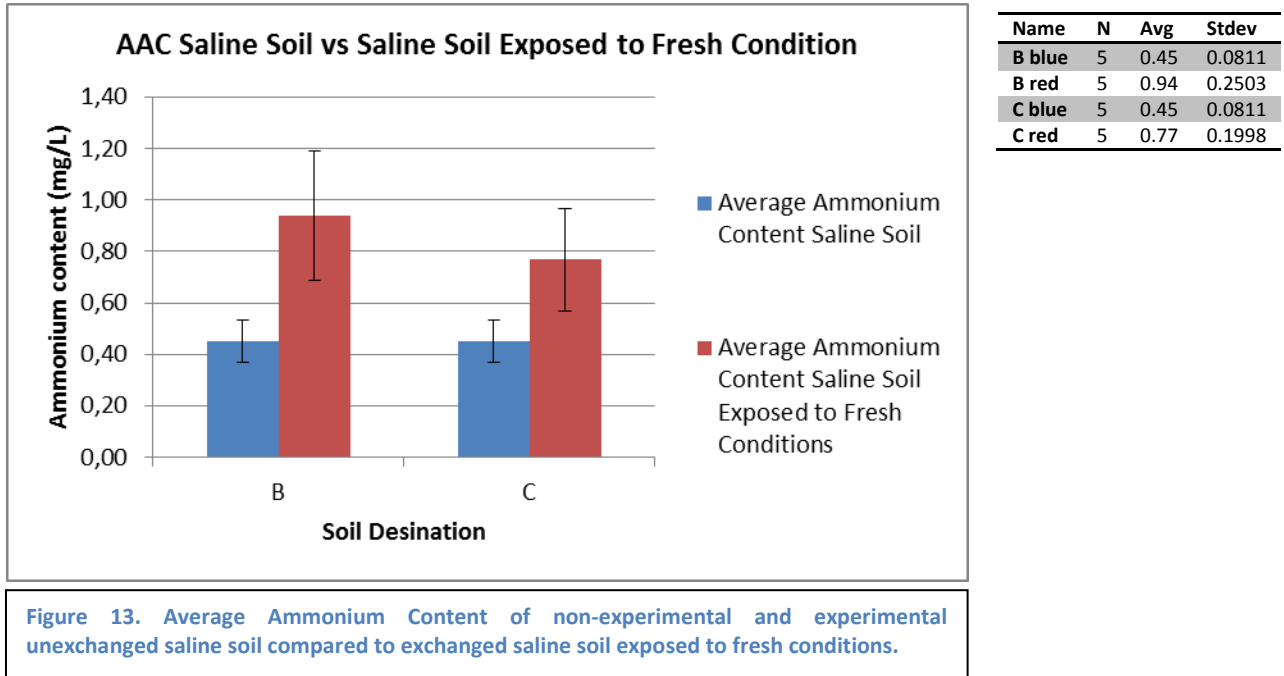
Figure 12. Pore water Average Ammonium Content of Fresh soil exposed to increased salinity.

The increased salinity did significantly reduce the average ammonium content of soil from site B (B  $\alpha=0.007$ ). The soil from site C however does not show a change in the average ammonium concentration when exposed to increased salinity (C  $\alpha=0.692$ ).



### AAC Exchange Salt to Fresh

Figure (13) bellow shows the effect of reduced salinity on the average ammonium content of the pore water in saline soil. Because the experimental verification test indicated that the non-exchanged experimental and non-exchanged non-experimental samples did significantly differ from each other they were not combined to enhance the group size.



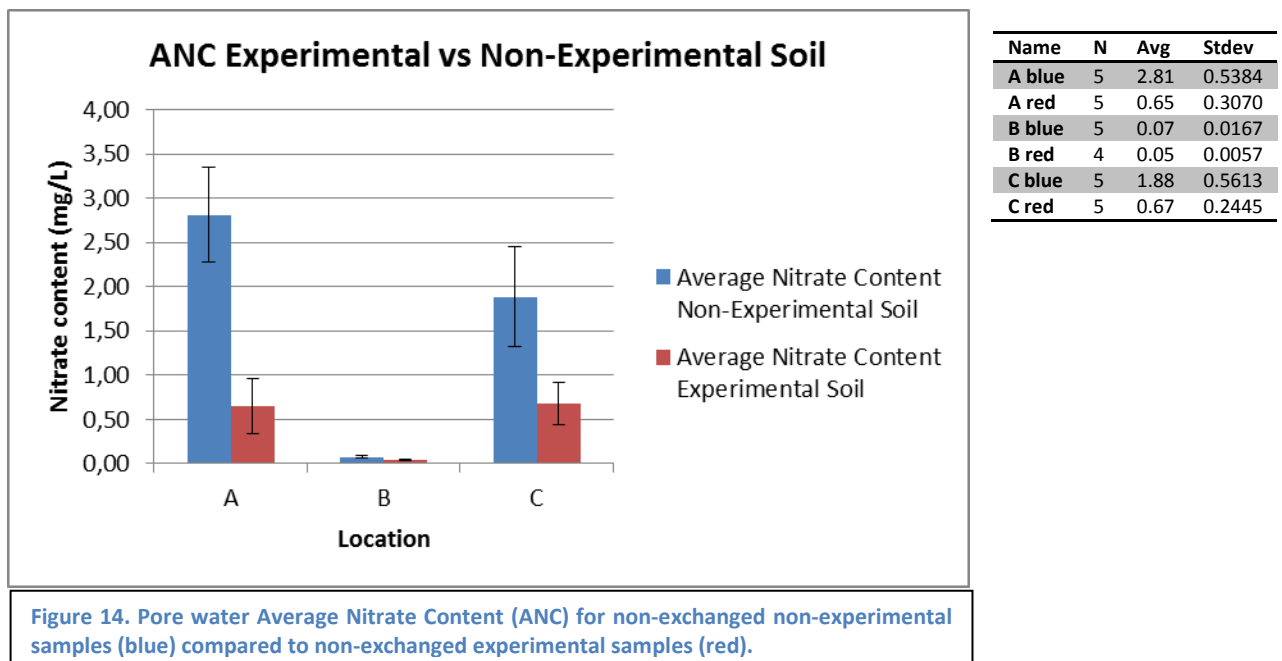
In both occasions a reduced salinity increased the ammonium concentrations significantly (B  $\alpha=0.009581$ , C  $\alpha=0.020343$ ).

## Nitrate

In contrary to the phosphorus and ammonium observations the pore water Average Nitrate Content (ANC) was measured using an ICP analyzer. Equal to the ammonium and phosphate twenty samples per site have been analyzed and the same tests were performed. Similar to the APC and AAC observations the low number of observations did not allow statistical methods to identify a possible outlier.

### ANC Experimental Verification

Similar to the AOC, APC and AAC observations the pore water average nitrate ( $\text{NO}_3^-$ ) content was also tested to verify the effects of the experimental setup. The results are shown in figure (14) below.



All experimental non-exchanged samples show a significant decrease of nitrate compared to the non-exchanged non-experimental samples. A  $\alpha=0.0014$ , B  $\alpha=0.0146$ , C  $\alpha=0.0180$ . Striking is the very low nitrate content on location B whereas the ammonium content was very high on this location.

### ANC Exchange Fresh to Fresh

Figure (15) below shows the pore water average ammonium content of the experimental non-exchanged fresh soil compared to the experimental exchanged samples exposed to the conditions on the alternate fresh site. The non-exchanged experimental fresh soil from site B (blue) is thus compared to the experimental soil from location B exposed to condition on location C and vice versa.

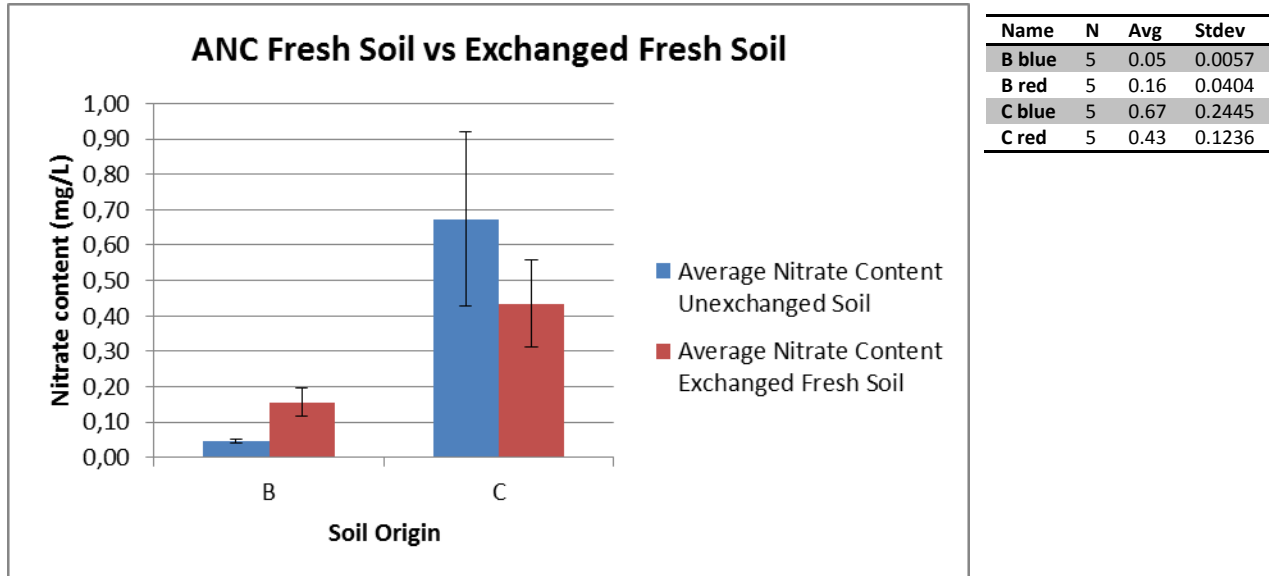


Figure 15. Pore water average nitrogen content of non exchanged experimental soil (blue) compared to exchanged experimental soil (red).

Due to the results in the experimental verification the non-exchanged experimental (blue) groups have not been enhanced with the non-experimental soil samples. The soil from location B exposed to the fresh conditions on site C shows a small but significant increase in pore water average nitrate content (B  $\alpha=0.003162$ ). Soil from site C exposed to the conditions on site B in contrary does not show a significant change in nitrate content (C  $\alpha=0.181503$ ).

### ANC Exchange Fresh to Salt

The figure below (16) displays the comparison between the pore water average nitrogen content of non-exchanged experimental fresh soil and experimental fresh soil exposed to increased salinity.

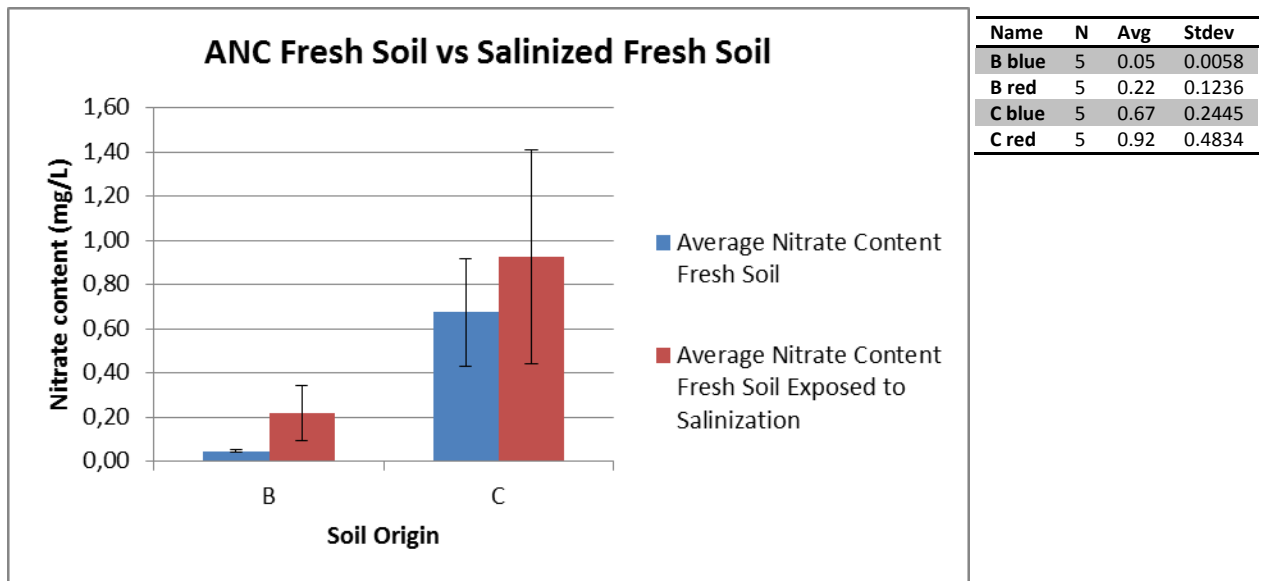


Figure 16. Pore water average nitrate content of non-exchanged experimental soil (blue) versus experimental fresh soil exposed to increased salinity (red).

The fresh soil from location B shows a slight but significant increase in nitrate content (B  $\alpha=0.028154$ ). Due to the high standard deviation the increase of pore water nitrate cannot be observed on soil from location C ( $\alpha=0.342401$ ).

### ANC Exchange Salt to Fresh

The final figure (17) shows the average nitrate content of the pore water extracted from experimental saline soil exposed to fresher conditions.

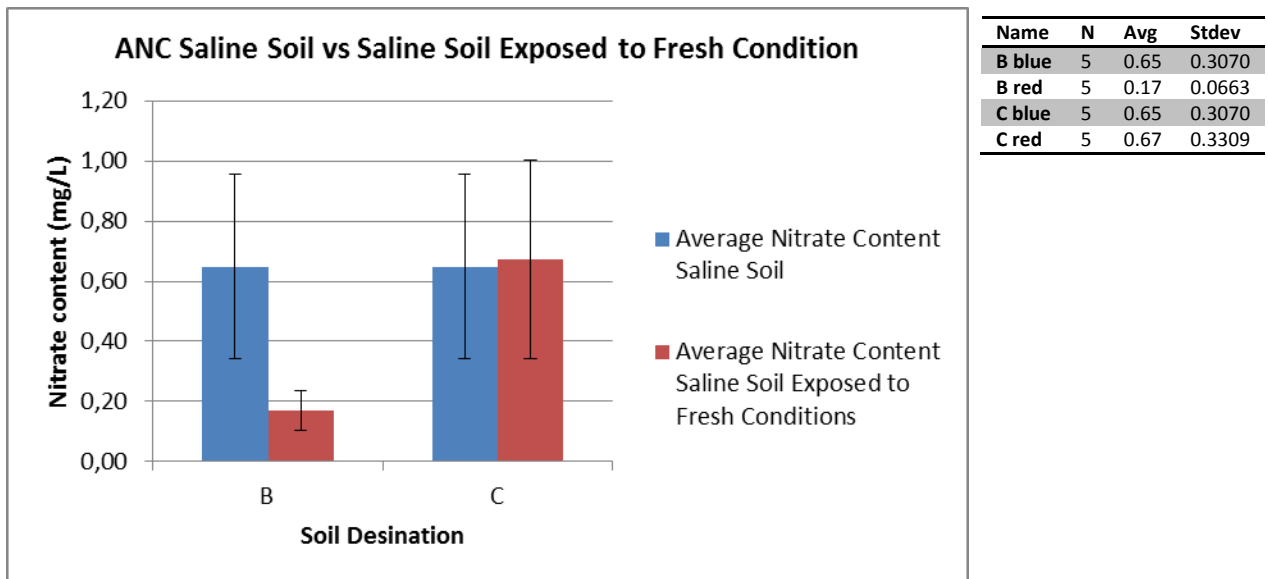


Figure 17. Pore water average nitrate content of experimental non-exchanged saline soil (blue) compared to the experimental saline soil exposed to fresh conditions (red).

The figure above shows the nitrate content of pore water from saline soil exposed to both the fresher conditions on location B and C. In the restored soil the nitrate content has significantly decreased (B  $\alpha=0.023677$ ), while on the natural soil no significant difference was observable (C  $\alpha=0.9007$ ).

## Summary of Statistical Result

The tables below serve as a brief summary of the experimental results displayed in the previous chapters. The letters correspond to the experimental locations from which the soil originates, except for the salt-fresh experiment where the letter corresponds to the location to which the soil was exposed. The = indicates no change observed, < relates to a reduction compared to the non-exchanged soil and > to an increase compared to the non-exchanged soil. The accompanying 'y' or 'n' indicates if the observed change is significant (y) or not significant (n).

<b>Organic Matter</b>	<b>A</b>		<b>B</b>		<b>C</b>	
Experimental verification	=	n	=	n	=	n
Fresh – Fresh			=	n	<	y
Fresh – Salt			=	n	=	n
Salt - Fresh			<	y	=	n

Table 5. Summary of AOC results.

The experiment did not change the soil organic content significantly on either of the locations. The natural fresh soil exposed to the low organic situation on the restored fresh site however lead to a significant decrease in organic matter. Exposure of natural and restored fresh soil to increased salinity did not significantly influence the AOC in contrary to the hypothesis. Exposure of saline soil to the fresh conditions on the natural site did also not yield statistical significant results. Exposure of saline soil to the restored location did result in a significant loss in organic matter. This observation is in line with the results from the exchange of natural fresh soil to the restored fresh location.

<b>Phosphate</b>	<b>A</b>		<b>B</b>		<b>C</b>	
Experimental verification	=	n	<	n	<	y
Fresh – Fresh			>	n	=	n
Fresh – Salt			<	y	<	y
Salt - Fresh			<	y	>	n

Table 6. Summary of APC results.

The experimental verification test shows a significant decrease in pore water phosphates in the restored and natural fresh wetlands. The exchange of natural fresh soil to the restored fresh site did not significantly alter the phosphorus content. The exchange in the alternate direction however shows an increase in phosphorus content.

<b>Ammonium</b>	<b>A</b>		<b>B</b>		<b>C</b>	
Experimental verification	>	y	>	y	>	n
Fresh – Fresh			=	n	>	y
Fresh – Salt			<	y	=	n
Salt - Fresh			>	y	>	y

Table 7. Summary of AAC results.

The ammonium observations indicate that the experiment leads to an increase of this substance in the pore water. This effect is also visible in the exchange between the natural and restored fresh sites and the exchange of saline soil to the either of the fresh locations. The fresh soil exposed to saline conditions shows a decrease in pore water ammonium content.

<b>Nitrate</b>	<b>A</b>		<b>B</b>		<b>C</b>	
Experimental verification	<	y	<	y	<	y
Fresh – Fresh			>	y	<	n
Fresh – Salt			>	y	>	n
Salt - Fresh			<	y	=	n

Table 8. Summary of ANC results.

The experiment decreased the pore water nitrate concentration significantly. Restored soil exposed to the natural conditions increases the nitrate concentration, while natural soil exposed to the restored location shows a non-significant reduction. Exposure of fresh soil to salinity increases the nitrate concentration though significant on the restored site and not-significant on the natural site.

## Discussion

This chapter is build up as following: First the experiment will be discussed pinpointing constraints and possible experimental flaws. The results are discussed in the same order as provided in the results chapter, including a possible explanation of the observed results and relate them to other researches.

### Experimental Discussion

As with most manipulative field experiments the environmental conditions are not controllable and thus subject to change. This study is no different in that: During the first two weeks of exposure frost may have reduced the active chemical and biological soil processes. Based on the found results it cannot be determined what the exact impact of these weather condition have been. Another problem of this fieldwork was the difficulty in finding suitable, representative and accessible locations in the Netherlands. The sites within the used locations are difficult to reach due to the regular flooding and muddy soil. This low accessibility limited the choice for experimental sites. The site selection was primarily based on the electric conductivity of the nearby river water and elevation compared to the high-tide mark. Preemptive screening of soil samples on the experimental sites could have been a useful addition in the site selection, allowing selection of location with more comparable soil chemistry.

The results indicate that the experiment was not entirely free from side-effects. Even though the 100mL plastic container in which the soil was exchanged and then buried was pierced it may still have retained water better than the surrounding non-experimental soil, thereby changing the experimental soil oxygen conditions. The exchange of the soil may have altered the structure which could also be of influence on the oxygen condition. Walking through the soil near the experiment may have affected the soil structure and may have increased local water retention. An effect that may also have influenced the observations is the non-homogenous distribution of observed chemicals in the soil. This, in combination with the low number of accessible experimental sites may have given an incomplete representation of the situation. In some occasions the low number of observations did not allow for clear exclusion of outliers and has increased the standard deviation making it more difficult to indicate significant results.



## Results Discussion

### Organic matter

The organic content experimental verification shows the expected result: the experiment did not alter the soil organic content significantly which supports the idea that the experimental and non-experimental soil are similar in regard to organic matter. The exchange between the fresh sites reduced the organic content in the soil from the natural location after exposure to the conditions on the restored site. This does not comply with the expectation that the conditions on both fresh sites are similar. A possible explanation for this result could be an unscreened soil attribute: oxygen availability.

The effect of salinization on fresh soil yields no significant results in contrary to the expected decrease in organic matter as suggested in research by Craft (2007) and Craft et al. (2008). An argument to contradict Crafts observations focuses on microbial life involved in the decomposition of soil organic carbon. Increased salinity is known to inhibit microbial activity and, depending on the microbial tolerance to salinity, causes death (Yan & Marschner, 2012). Without these microbes organic decomposition would be lower than with them since they do not break down any organics, which would eventually result in increasing organic content. Capone & Kiene (1988) however support Craft by indicating that sea-salt supplied sulfate changes the organic matter mineralization pathways from methanogenesis to faster sulfate-reduction, especially in anaerobic conditions. Doddema et al. (1985) show that organic matter in salinized soils can briefly act as a buffer to the osmotic shock, allowing temporal regular production and decomposition and thus result in no observable difference. Setia et al. (2011) also support Craft et al. (2008) and adds to his argument that increased salinity is likely to reduce local production of organic matter. The brief experimental exposure limited the effects of local production in this case but it would mean a longer exposure to increased salinity yields different results and would allow more accurate extrapolation to future seasonal salinity alterations. The previously mentioned limited timeframe of the experiment and the low decomposition speed due to cold weather conditions may also have influenced the found results.

Exposure of saline soil has shown a significant decrease when exposed to the conditions on the restored fresh wetland, exposure to the natural TFW did not significantly alter the organic content. These observations are in line with the previously shown results on the exchange of fresh soil to the alternate fresh site and supports the idea that the conditions on location B increase carbon mineralization. A possible explanation could be in the limited availability of organic matter in the restored location leading to more competition and results in faster decomposition. No literature was found that describes a possible link between the decomposition speed of organic matter and high ammonium availability. The high ammonium and low nitrate concentrations in the restored wetland however are an indication for more constant anaerobic conditions which are known to have an accelerative effect on decomposition (Sutton-Grier et al. 2011) and may increase the release of nitrate (Capone & Kiene, 1988).

## Phosphate

The experimental verification shows that the experiment did not significantly alter the pore water phosphate content on location A and B, but decreased it on location C. The presence of a suspected outlier on location B could indicate that the phosphate content can vary a lot on a small spatial scale. This local variation and the limited sample size could thus also explain the difference between the experimental and non-experimental observations on location C. Another possible explanation for the reduction in the amount of phosphate on the natural site is that it could be the result of an alteration in the soil structure by the experiment which allowed phosphates to leech more easily.

The exposure of restored fresh soil to the conditions on the natural freshwater wetland also suffers from a high standard deviation which makes analysis difficult. A possible explanation for the phosphate increase on this soil could be the leaching of surrounding pore water into the sample or may be, as earlier mentioned, due to local variations.

The effect of increased salinity on the pore water of fresh soil samples shows a distinct significant decrease on both the natural and restored location. This observation is in line with experiments by Jun & Craft (2012, to be published) and Reddy et al. (1999). The main mechanism for the loss of phosphates from the pore water in floodplains is thought to be aluminum and iron reduction (Darke & Walbridge, 2000). Another possibility that cannot be excluded based on the observations presented in this research is leaching: an altered ion-exchange due to a salinization-induced change in the CEC (cation-exchange complex) could release phosphates allowing them to leech. To verify increased sorption by ion exchange or reduced metals as a main cause for the loss of phosphate from the pore water, an analysis of soil particles such as described by Ruttenberg (1992) and Slomp et al. (1996) is required.

The experiment where saline soil is transported to both fresh sites indicates no change on the natural site and a reduction in the pore water phosphate concentration on the restored site. The lack of change on the natural site does not comply with the hypothesis that reduction of salinity allows the soil to release sorbed phosphates but points towards another mechanism. As mentioned before increased salinity reduced the pore water phosphate content on both locations, thereby making it unlikely that a soil property such as oxygen availability is a main reason for a reduction in the pore water phosphate concentration. A salinity induced change in the CEC would release phosphates allowing them to leech from the soil, permanently removing them from the system. The soil would then have to 'recharge' the phosphate content which likely did not happen within the experimental timeframe.

### Ammonium & Nitrate

The soil nitrogen cycle is complex and since nitrate molecules can be transformed into ammonium (ammonification) the observations are also linked. Therefore both groups of observed results are combined in one discussion.

The experimental verification showed a significant increase in the amount of ammonium in the pore water on location A and B, on location C the ammonium content was also increased but the results do not show a significant difference. A possible explanation is the increased decomposition of organic matter which releases nitrates. Another explanation for this increase is an alteration of the soil oxygen conditions due to experimental interference and a quick adaptation to anaerobic ammonification of soil nitrate. Antheunisse et al. (2006) describes similar results in a comparable one-year field experiment: nitrate mineralization rates increased under anoxic conditions, while denitrification was not so much affected. Exposure of the high ammonium restored soil to the low ammonium natural fresh location did also increase the ammonium concentration significantly. This could be explained by Mahrous et al. (1983) who also investigated the effect of soil oxygen conditions on nitrogen cycles and linked them to the decomposition of organic matter: Anaerobic soils with high organic matter show more ammonification of nitrate than soils with low organic content, in line with this research observations for organic matter, the observed nitrate decrease and ammonium increase. The observed nitrate reduction under the experimental conditions supports this theory. It however remains possible that the low number of samples or an experimental flaw biased the results: anaerobic denitrification of Nitrate to  $N_2$  would be a very likely explanation for the observed nitrate decrease since denitrification is energetically favorable above ammonification. Future research should thus focus on the effects of organic matter on nitrification, denitrification, determining the importance of denitrification and ammonification for nitrate reduction and the influence of organic matter as a source of nitrate.

The results from the exposure of the low ammonium natural fresh soil from location C to the restored site also show a significant ammonium increase. A possible explanation here could be the leeching of ammonium from the surrounding soil. Another explanation, supported by Mahrous et al. (1983) and Antheunisse et al. (2006) and previously discussed observations, is that the restored location or the experiment made the samples more anaerobic increasing the ammonification of available nitrate. Again the anaerobic ammonification theory is supported by the nitrate observations: natural fresh soil exposed to the restored conditions reduced the pore water nitrate content. Another possibility is the release of nitrogen from organic matter due to increased mineralization.

Exposure of restored fresh soil to increased salinity shows a significant ammonium decrease. The natural soil in contrary shows no significant ammonium change when exposed to higher salinity. The pore water AAC reduction on the restored location can be explained by the quick release of sorbed ammonium to the water column allowing it to leech. Jun & Craft (2012, to be published) found a similar result: soils of tidal floodplain forests exposed to increased salinity also released ammonium. They also found results that suggest that this process is enhanced by low oxygen conditions. The lack of observable difference in ammonium concentration on the natural site after exposure to salinity also fits their conclusion: The situation in the natural TFW is likely more aerobic. Following Jun & Craft this results in a lower ammonium desorption from the soil.

The initial low ammonium concentration did not alter by the experiment. The increased salinity may however have altered the soil conditions for ammonifying bacteria reducing their activity even further (Yan & Marschner, 2012). Peelen (undated) and Yan & Marschner (2012) indicated microbial activity can be inhibited by increased salinity. Adding to Mahrous et al. (1983) they suggest that increased salinity could inhibit nitrate decomposition and respiration. This explains the increased nitrate levels in the restored and natural soils.

The exposure of saline soil to the natural and restored fresh locations increases the pore water ammonium concentration significantly. In this case multiple factors could explain the observed results: reduced salinity may improve conditions for ammonifying bacteria which however is not in line with the observations in the increased salinity experiments. On the other hand the oxygen conditions could play a role in the ammonium increase. The soil state (natural or restored) did not influence the observations. The pore water nitrate decreased significantly upon exposure to fresher conditions, an observation which is in line with the ammonium increase and supports the ammonification theory. As Weston et al. (2010) describe ammonium could be released from the soil due to an increased ionic strength of the water. However the low observed organic matter decomposition indicates that the release of nitrate from organic matter, as was described by Craft (2007) and Weston et al. (2006) is relative small and cannot compensate the reduction due to anaerobic dissimilation or ammonification processes. Based on the found results it is not possible to pinpoint the exact source of the increased nitrate concentration in the desalinated soils.

### **General Discussion**

In the introduction the hypothesis was formulated that increased salinity would increase organic carbon mineralization. In contrary to this hypothesis organic matter did not decrease under salinization, therefore this hypothesis must be rejected. However the constraints of the experiment may have influenced this result. The second hypothesis theorized that increased salinity would increase the soil nitrate release, increasing the pore water nitrate content. Based on the results found this hypothesis can be accepted with a note that soil characteristics may be of influence. Thirdly increased salinity hypothesized to remove phosphate from the pore water and sorb to soil particles. A decreased pore water phosphate concentration was indeed observed, yet additional research is required to pinpoint where to.

No literature was found that describes the effects of desalinization in TFWs, however based on the description that TFW's are highly resilient ecosystems it was to be expected that a reduction of salinity will allow the ecosystem to recover. Hence desalinization would slow the carbon mineralization process down leading to a gradual increase in the soil organic carbon content. This however was not observed; the restored soil showed a decrease in the amount of soil organic carbon under desalinization while no significant change was found after exposure to the natural TFW. As stated this is likely due to the fact that organic matter regeneration depends mostly on local production. Desalinization was also hypothesized to re-increase the nitrogen retention and release of sorbed phosphates: the pore water ammonium concentration increased further under desalinization while the pore water nitrate content slightly decreased. Based on these observations this hypothesis is also to be rejected. The pore water phosphate concentration reduced under desalinization in the restored wetland and increased in the natural TFW. It can thus be stated that future research is needed for a definitive rejection of this hypothesis.

## Conclusion

To recap the main question of this research was

*What are the effects of salinization and desalinization on tidal freshwater wetland available soil nutrients and organic content?*

In order to answer this question the following sub questions have been answered

*What are the effects of salinization on the availability of nutrients in tidal freshwater wetland soils?*

Fresh soil exposed to increased salinity shows a significant decrease in the amount of phosphate in the pore water induced by the increased ionic strength of the saline water. Anaerobic conditions and resulting Iron and Aluminum reduction could also be important factors in phosphate reduction, yet more specific research on this topic is required. The restored site shows a significant increase in the amount of ammonium when exposed to higher salinity while the natural site does not significantly change. The pore water nitrate concentration in the restored soil declined significantly after exposure to salinity, while the nitrate concentration in the natural site did not alter. A likely explanation for the latter is a salinity induced inhibition of decomposing nitrate bacteria.

*What is the effect of salinization on the amount of organic matter in tidal freshwater wetland soils?*

Against the expectation the amount of organic matter in the soil did not significantly decrease after exposure to increased salinity. Weather conditions, the relative short experiment and local production or deposition are possible explanations to this observation.

*What is the effect of desalinization on the availability of nutrients in salinized tidal freshwater wetland soils?*

Saline soils exposed to fresh conditions have shown mixed results. Exposure of saline soil to the restored fresh conditions shows a significant decrease in the amount of phosphate in the pore water, induced by a changed CEC. The same soil exposed to the natural fresh site indicates a slight, non-significant increase. In this case the more aerobic conditions are an explaining factor. The amount of ammonium in the pore water increased significantly due to a changed CEC, while the nitrate concentration was reduced due to recovering anaerobic ammonification.

*What is the effect of desalinization on the amount of organic matter in salinized tidal freshwater wetland soils?*

Exposure of saline soil to the restored fresh site reduced the amount of organic matter in the soil while the natural fresh site did not significantly alter the organic content. Differences in the chosen locations are likely to have influenced these results.

In general the conclusion can be drawn that future salinization and desalinization in tidal freshwater wetlands is likely to result in an increased nitrogen load in downstream estuaries and adjacent coastal areas.

## Suggestions

Future research on this topic should aim to verify the found results. Specific focus should be put on acquiring results from more sites from multiple estuaries and river systems. It may also be beneficial to do more in-detail analysis of soils chemistry in order to determine the most important active processes and pinpoint specific soil ammonium binding chemicals and quantify the effects of reduced local production of organic matter on the TFW nitrogen cycle.

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## Appendix A, Organic Content Data

Label	% OC	Label	% OC	Label	% OC	Label	% OC	Label	% OC	Label	% OC
AA1x1	11,90476	AB11	3,185925	BB1x1	4,372093	BA11	12,5	CC1x1	13,79039	CA11	12,72085
AA1x2	13,69048	AB12	3,416624	BB1x2	4,231796	BA12	9,172809	CC1x2	14,0457	CA12	13,35559
AA2x1	12,19839	AB21	3,529412	BB2x1	3,908139	BA21	7,778469	CC2x1	14,72019	CA21	12,59105
AA2x2	12,55061	AB22	3,890675	BB2x2	3,892313	BA22	6,954689	CC2x2	14,7016	CA22	13,89222
AA3x1	11,65138	AB31	7,908612	BB3x1	3,562276	BA31	8,445642	CC3x1	17,26535	CA31	11,08165
AA3x2	11,56337	AB32	5,021317	BB3x2	3,924647	BA32	9,731013	CC3x2	14,7806	CA32	12,16703
AA4x1	13,6141	AB41	3,702372	BB4x1	4,352227	BA41	8,042771	CC4x1	15,18114	CA41	12,02723
AA4x2	15,41756	AB42	3,703704	BB4x2	4,82977	BA42	7,87949	CC4x2	15,17184	CA42	11,94719
AA5x1	12,38698	AB51	3,492402	BB5x1	3,308102	BA51	7,274401	CC5x1	15,00132	CA51	13,70968
AA5x2	11,31579	AB52	3,74415	BB5x2	3,655565	BA52	10,9017	CC5x2	15,8202	CA52	13,90552
AA6x1	9,898629	AB61	4,843517	BB6x1	3,353057	BA61	9,237668	CC6x1	15,21555	CA61	14,07529
AA6x2	10,30195	AB62	3,967855	BB6x2	3,512623	BA62	8,80139	CC6x2	14,47049	CA62	12,78317
AA7x1	12,69297	AB71	2,723615	BB7x1	3,382621	BA71	5,081179	CC7x1	16,46612	CA71	11,64912
AA7x2	12,5	AB72	3,403756	BB7x2	3,531838	BA72	4,631696	CC7x2	16,51584	CA71	12,39946
AA8x1	13,04945	AB81	3,550974	BB8x1	3,338335	BA81	9,648712	CC8x1	16,60517	CA81	13,59833
AA8x2	15,71872	AB82	3,005865	BB8x2	3,353803	BA82	10,33082	CC8x2	15,40881	CA82	14,65457
AA9x1	13,24415	AB91	4,492441	BB9x1	3,09842	BA91	10,29193	CC9x1	15,58538	CA91	11,21055
AA9x2	14,12429	AB92	4,125737	BB9x2	3,463522	BA92	8,100358	CC9x2	13,60294	CA92	11,58672
AA10x1	11,23529	AB101	4,3654	BB10x1	3,607748	BA101	8,641975	CC10x1	15,54404	CA101	12,60339
AA10x2	11,83013	AB102	3,883495	BB10x2	3,730548	BA102	7,47752	CC10x2	17,55006	CA102	13,1766
AA11	14,15094	AC11	14,25598	BB11	4,058442	BC11	10,09047	CC11	13,6896	CB11	3,972536
AA12	11,26255	AC12	14,21769	BB12	3,095357	BC12	8,791209	CC12	13,60882	CB12	3,28201
AA21	14,50285	AC21	14,53287	BB21	4,128788	BC21	12,51101	CC21	14,72785	CB21	4,72103
AA22	14,98944	AC22	14,81976	BB22	3,903016	BC22	14,19118	CC22	14,71119	CB22	4,283657
AA31	13,53747	AC31	15,311	BB31	2,827068	BC31	10,90487	CC31	15,77855	CB31	7,108042
AA32	13,21696	AC32	15,70415	BB32	3,244755	BC32	12,38338	CC32	14,61187	CB32	3,566466
AA41	13,61842	AC41	14,61737	BB41	3,5155	BC41	11,65591	CC41	13,0064	CB41	4,425323
AA42	13,68421	AC42	13,86139	BB42	2,950522	BC42	10,33333	CC42	14,67305	CB42	5,244755
AA51	16,07551	AC51	16,09687	BB51	2,999294	BC51	12,56798	CC51	14,12352	CB51	3,134182
AA52	14,96774	AC52	15,07618	BB52	2,792506	BC52	12,25225	CC52	14,39342	CB52	4,950495
AA61	14,6323	AC61	14,24936	BB61	4,089616	BC61	12,63001	CC61	12,6506	CB61	4,444444
AA62	15,89744	AC62	14,30746	BB62	4,046013	BC62	10,85627	CC62	13,54642	CB62	3,914178
AA71	14,04287	AC71	14,78521	BB71	3,374908	BC71	10,37234	CC71	13,78433	CB71	3,817992
AA72	11,26154	AC72	19,54023	BB72	3,63047	BC72	12,03288	CC72	14,07547	CB72	3,919631
AA81	13,76712	AC81	13,93728	BB81	3,959753	BC81	14,44653	CC81	13,55932	CB81	4,757976
AA82	12,24018	AC82	13,56467	BB82	4,295533	BC82	13,92857	CC82	13,77662	CB82	3,979366
AA91	10,60071	AC91	14,25689	BB91	3,074324	BC91	12,61312	CC91	13,41176	CB91	3,572388
AA92	10,6626	AC92	14,62687	BB92	3,316032	BC92	10,78963	CC92	13,84248	CB92	3,096825
AA101	17,59425	AC101	13,12027	BB101	3,357426	BC101	11,78271	CC101	13,26894	CB101	2,962298
AA102	16,21622	AC102	13,39066	BB102	3,163235	BC102	11,49194	CC102	13,09524	CB102	3,444564

### Legend Example AA1x1

- The first letter of the code states the soil destination to which the sample has been exposed
- The second letter states from which location the soil originates.
- The third number refers to the soil sample number
- The x marks samples without experimental influence, the so-called full-soil samples. Absence of an x is an experimental sample
- Since the organic matter was determined in duplo the last number marks which of the two runs the sample was taken.

## Appendix B, Phosphate Content data

Label	PO4 umol/L	PO4 mg/L	Label	PO4 umol/L	PO4 mg/L
AA1x	1,0	0,09	BC1	1,8	0,17
AA2x	0,9	0,09	BC2	1,9	0,18
AA3x	1,4	0,14	BC3	2,2	0,21
AA4x	1,3	0,12	BC4	5,5	0,52
AA5x	1,5	0,15	BC5	0,7	0,07
AA1	2,0	0,19	CC1x	3,8	0,36
AA2	0,7	0,06	CC2x	3,0	0,29
AA3	1,1	0,11	CC3x	3,7	0,35
AA4	1,1	0,10	CC4x	3,5	0,34
AA5	1,0	0,10	CC5x	3,6	0,34
AB1	1,5	0,14	CC1	2,2	0,21
AB2	1,1	0,10	CC2	1,7	0,16
AB3	0,7	0,07	CC3	2,0	0,19
AB4	0,4	0,04	CC4	1,3	0,13
AB5	1,4	0,13	CC5	1,6	0,16
AC1	2,5	0,23	CA1	1,9	0,18
AC2	2,3	0,22	CA2	0,7	0,07
AC3	2,0	0,19	CA3	1,1	0,10
AC4	1,2	0,11	CA4	1,8	0,17
AC5	1,8	0,17	CA5	1,6	0,15
BB1x	2,4	0,23	CB1	13,3	1,26
BB2x	1,0	0,10	CB2	1,6	0,16
BB3x	2,5	0,23	CB3	4,6	0,44
BB4x	5,0	0,47	CB4	7,9	0,75
BB5x	0,9	0,09	CB5	7,9	0,75
BB1	0,8	0,07			
BB2	0,9	0,08			
BA1	0,8	0,08			
BA2	0,5	0,05			
BA3	0,4	0,03			
BA4	0,4	0,04			
BA5	0,4	0,04			

### Legend Example AA1x1

- The first letter of the code states the soil destination to which the sample has been exposed
- The second letter states from which location the soil originates.
- The third number refers to the soil sample number
- The x marks samples without experimental influence, the so-called full-soil samples. Absence of an x is an experimental sample

### Appendix C, Ammonium Content data

Label	NH4 umol/L	NH4 mg/L	Label	NH4 umol/L	NH4 mg/L
AA1x	7,7	0,14	CC1x	4,0	0,07
AA2x	5,7	0,10	CC2x	9,0	0,16
AA3x	8,1	0,15	CC3x	7,4	0,13
AA4x	7,0	0,13	CC4x	5,4	0,10
AA5x	4,3	0,08	CC5x	7,6	0,14
AA1	32,0	0,58	CC1	15,3	0,28
AA2	26,7	0,48	CC2	40,5	0,73
AA3	20,5	0,37	CC3	40,6	0,73
AA4	23,7	0,43	CC4	14,3	0,26
AA5	22,3	0,40	CC5	15,3	0,28
AB1	90,8	1,64	CA1	49,2	0,89
AB2	130,4	2,35	CA2	23,8	0,72
AB3	85,3	1,54	CA3	51,5	0,93
AB4	48,8	0,88	CA4	47,8	0,86
AB5	76,7	1,38	CA5	24,3	0,44
AC1	20,1	0,36	CB1	157,6	2,84
AC2	19,5	0,35	CB2	148,7	2,68
AC3	29,4	0,53	CB3	154,4	2,79
AC4	22,2	0,40	CB4	209,7	3,78
AC5	53,2	0,96	CB5	176,4	3,18
BB1x	119,8	2,16			
BB2x	81,3	1,47			
BB3x	104,3	1,88			
BB4x	126,2	2,28			
BB5x	108,6	1,96			
BB1	201,1	3,63			
BB2	141,8	2,56			
BA1	40,1	0,72			
BA2	54,6	0,99			
BA3	57,1	1,03			
BA4	37,2	0,67			
BA5	71,4	1,29			
BC1	97,3	1,76			
BC2	141,8	2,56			
BC3	92,1	1,66			
BC4	109,8	1,98			
BC5	123,5	2,23			

#### Legend Example AA1x1

- The first letter of the code states the soil destination to which the sample has been exposed
- The second letter states from which location the soil originates.
- The third number refers to the soil sample number
- The x marks samples without experimental influence, the so-called full-soil samples. Absence of an x is an experimental sample

### Appendix D, Nitrate Content data

Label	NO3 mg/L	Label	NO3 mg/L
AA1x	2,21	CC1x	1,76
AA2x	2,25	CC2x	1,69
AA3x	3,05	CC3x	1,81
AA4x	3,22	CC4x	2,83
AA5x	3,32	CC5x	1,33
AA1	1,06	CC1	0,85
AA2	0,25	CC2	0,4
AA3	0,5	CC3	0,54
AA4	0,81	CC4	0,58
AA5	0,62	CC5	1
AB1	0,11	CA1	0,38
AB2	0,11	CA2	0,28
AB3	0,4	CA3	0,75
AB4	0,28	CA4	0,94
AB5	0,19	CA5	1,02
AC1	1,64	CB1	0,16
AC2	0,47	CB2	0,2
AC3	1,09	CB3	0,12
AC4	0,49	CB4	0,11
AC5	0,93	CB5	0,19
BB1x	0,1		
BB2x	0,07		
BB3x	0,06		
BB4x	0,08		
BB5x	0,06		
BB1	0,04		
BB2	0,05		
BA1	0,14		
BA2	0,28		
BA3	0,18		
BA4	0,14		
BA5	0,11		
BC1	0,54		
BC2	0,48		
BC3	0,46		
BC4	0,47		
BC5	0,22		

#### Legend Example AA1x1

- The first letter of the code states the soil destination to which the sample has been exposed
- The second letter states from which location the soil originates.
- The third number refers to the soil sample number
- The x marks samples without experimental influence, the so-called full-soil samples. Absence of an x is an experimental sample