Stepping stones towards saline organic production systems

Saline organic, final report

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Content

1 Introduction

1.1 The world food situation

Whereas the worldwide population continues to grow, in 2017 the FAO described a rise in world hunger, returning to levels that prevailed almost a decade ago (FAO *et al*., 2018). There has been a further increase in the percentage of people having insufficient dietary energy consumption in 2017. The FAO describe increased climate variability as one of the three key factors driving this recent rise in global hunger. Salinization of agricultural land is one of the processes resulting from the changes in weather and climate, and increasing world hunger.

1.2 Need for adaptation and sustainability

Increasing sustainability in food production therefore represents a multiple-win situation. Firstly, it lowers the direct effects of the food production on increasing greenhouse gas emissions and climate change. Secondly, it is essential the preserve ecosystem health, which is vital to food production. Thirdly, concerning saving water and other external inputs, it can even enlarge the possible food production level in the world. Finally, change towards sustainable food production could (and probably would) also result in a shift towards a more healthy diet. We envisage several ways to increase sustainability:

1. Further develop low input agricultural production systems, from which organic agriculture is one of the most important. As shown by Gomeiro *et al.* (2008) these can result in less energy demand and CO_2 abatement. Important environmental and social services will be promoted (e.g. preserving and improving soil quality, increasing carbon sink, minimizing water use, preserving biodiversity, halting the use of harmful chemicals).
2. A shift towards more plant-based diets. Plant-based diets use much less natural resources and emit less greenhouse gasses (Sabaté and Soret 2014; van de Kamp *et al*. 2018). A large part of the population would need to shift towards a lower meat and dairy product consumption, and more diverse diet containing – plant-based products would be very helpful in this transition.
3. One of the main limiting resources is water. Water footprints of more healthy diets, especially those with less or without meat are substantially smaller (30-55 %) than with meat (VanHam *et al*. 2018) Therefore, also for this resource a change towards a more plant-based diet is positive.

1.3 Saline organic agriculture as a chance

As pointed out by Rozema and Flowers (2008), only 1% of the water on Earth is fresh. Agriculture is one of the large users of this resource. Strikingly, there is just as much brackish water as there is freshwater. The remaining 98% of the water on earth is seawater. Salinization has been increasing during the last decades globally (Ivushkin *et al*., 2019; United nations University, 2014). Enabling agriculture to include the use of brackish water, or even seawater, would enormously help towards its sustainability. Combined with a shift towards organic production techniques and the widening of the range of edible plants, this would be a great step forward towards a sustainable future.

For organic agriculture the development of saline crops presents a particular challenge. In organic crop rotations, green manures (legumes) are essential for fixing the nitrogen necessary to maintain a reasonable productivity. However, many green manures are sensitive to salinity, and also the nodulation process is often stated to be highly sensitive to salt stress, although there is room for selection (Zahran, 1999). Cultivars of some species (*Medicago sativa, Melilotus officinalis*) have shown some potential in artificially controlled saline field conditions (Bruning et al., 2015). Their practical application in a crop sequence in saline soils has yet to be studied. Therefore development of a salt tolerant green manure cultivar, that can fix nitrogen in saline soils for a following crop, is a crucial factor for organic saline agriculture

2 Aims and hypotheses

At present, there is a growing list of potential salt tolerant cultivars for the main stream food chain, and of plant species for potentially new niche crops. This list includes for example salt tolerant potato cultivars, beet cultivars, cereal cultivars and quinoa for main stream crops and for example sea beet, seakale, Salicornia, iceplant (*Mesembryanthemum crystallinum*) and many others as potential small scale crops. Included are candidate salt tolerant green manure cultivars.

In this research we aim to study four aspects crucial for the development of a sustainable organic production under saline conditions:

- The consumers perspective and demands in the contexts of specific diets, in terms of type and quality of saline crops.
- Field performance of these crop species (including green manures) in a system-context under saline conditions in two soil types in the Netherlands, with a focus on sustaining soil quality.
- Field performance of candidate green manure species, that are essential in providing nitrogen for organic saline crop rotations.
- Comparing potential quality aspects of the produce for dietary requirements taking into account management aspects.

Hypotheses to be tested

1. Saline crops are promising additives within plant-based diets
2. Saline soil conditions require adapted crop rotations based on salt adapted crop species to be truly sustainable for these areas.

3. Salt tolerant green manure cultivars can be selected to enable organic saline rotations.
4. Saline conditions require adapted crop management to enable sustainable production and long term soil fertility.

3 Literature study: comparing salt tolerance in research

Within the scientific literature there are many different units with which the salinity of the soil is indicated. A first step was to combine the different units indicative for salinity into one table that would allow comparison (Table 1). In this report ds/m paste, the electric conductivity of saturated soil paste (ECe) was chosen as the main unit. This was done because it is widely used in research, and up to some level it is standardized for comparing effects on plants or crops: all soils wetted up to field capacity (saturated paste) before making the measurement. It therefore overrides differences in soil porosity.

Table 1. Different units used to quantify salinity In this report, ECe, or the EC of the saturated paste, in dS/m is the unit used for salinity level.

dS/m	ppm	NaCl gram/l	Cl mg/l	mmol NaCl
1	640	0,6	151	11
2	1280	1,3	374	22
4	2560	2,6	928	44
8	5120	5,1	2302	88
12	7680	7,7	3915	132
16	10240	10,2	5707	175
20	12800	12,8	7644	219
30	19200	19,2	13002	329

A second step was determining when a crop is considered sensitive to saline soil. For this we used the classic classification of soil salinity (Ivushkin *et al*., 2019; FAO, Table 2) which is quite comparable to that of Maas (1990). The levels of salinity are classified according to the performance of crops.

Table 2. Soil salinity classes (Ivushkin et al., 2019; FAO): (http://www.fao.org/3/x5871e/x5871e04.htm).

ECe (dS/m)	Salinity level	Crop class
0-2	Non-saline	Only small effects
2-4	Slightly saline	Sensitve crops are restricted
4-8	Moderately saline	Many crops are restricted
8-16	Strongly saline	Only tolerant crops
>16	Very strongly saline	Only very tolerant crops

Ivushkin *et al*. (2019) also gives insight in de distribution of saline soils (Table 3). They show that 97% of the soils that have saline conditions are only slightly saline (up to 4 dS/m), and another 2% are moderately saline. In 2016, this concerned 1061 Mha of soil, with an increase of around 17 Mha in the last thirty years.

Table 3. The world salt affected area as predicted from ground truth data, thermal satellite imagery and soil property maps for different years, Mha

in Mha	**Slightly** saline	**Moderately** saline	**Highly** saline	**Extremely** saline
ECe, ds/m	**2–4**	**4–8**	**8–16**	**>16**
May/86	878	30	2	5
May/00	810	28	4	8
May/02	920	20	3	5
May/05	888	22	2	6
May/09	1028	20	2	7
May/16	1036	25	3	6
% of Total				
May/16	97	2	0	1

Most plants differ in salt sensitivity is their different phases, where the seedling phase is often most sensitive and the cropping phase is least sensitive. In literature, either the salt tolerance is measured and reported in the seedling phase, or in whole crops growing in saline fields (field performance).

For the seedling phase, experiments are often performed in petri-dishes and pots with a consistent environment, so more or less controlled conditions. During field tests, either seeds are sown or sprouts are transplanted into previously fresh water irrigated fields and are then irrigated with salt water. In most of these field tests, the environment is less consistent as are the results, although they give a better indication of real situation than looking only at seedlings.

Classical theory on salt tolerance describes the relation between plant growth or yield and salinity level in the soil as a linear line starting at a threshold, and then going down with a certain slope (Fig. 1, adapted from van Dam *et al*., 2007). One of the characteristics differentiating between sensitive and salt tolerant crops, that is often used in literature, is this threshold level of salinity, at which a clear decrease in yield starts. The slope is another factor, however it is much more difficult to measure and therefore less indicative. In our longlist and selection of potential interesting salt tolerant crops, we therefore firstly focussed on the threshold values of crops as one of the factors to take into account.

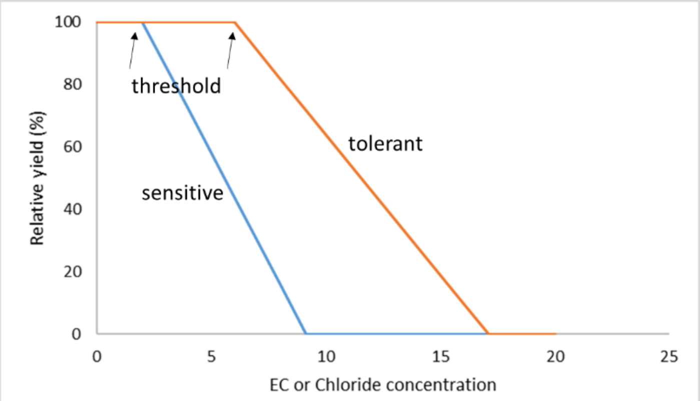

Figure 1. Representation of the classical theory describing the reaction of crops to saline conditions.

In Table 4 a number of halophyte species that are described as eatable and in some cases used as crops is shown. Some of these can fixate nitrogen through symbiosis, however these are woody plants and need a number of years to develop. Most of the produce of these halophytes are leafs and sometimes small amounts of seeds. Most of these plants will however grow under high levels of salinity.

Table 4. A list of halophyte plants used as crop. Plant that fixate nitrogen in symbiosis with bacteria are marked green.

Common name	**Latin name**	**type of crop**
batis (seawort)	*Batis maritima*	tropical, seeds with starch, no commercial use
chinese liquorice	*Glycyrrhiza uralensis*	woody plant, roots
common glasswort	*Salicornia europaea*	leaf crop
common ice plant	*Mesembryanthemum crystallinum*	leaf crop
goji berry	*Lycium barbarum*	woody plant, berries
liquorice	*Glycyrrhiza glabra*	woody plant, roots
orache	*Atriplex hortensis*	leafs, seeds, no commercial use
pickleweed	*Salicornia bigelovii*	leaf crop
samphire	*Crithmum maritimum*	leaf, herb
sea aster	*Aster tripolium / Aster pannonicus*	leaf crop
sea beet	*Beta vulgaris subsp. maritima*	leaf crop
sea kale	*Crambe maritima*	leaf crop
sea-buckthorn	*Hippophae rhamnoides*	woody plant, berries

Table 5 presents a list of many other crops, with their threshold value for salt tolerance. In the first column, arable non-N fixing crops are listed. Most of them are quite sensitive to saline conditions. Exceptions are pursley (or purslane, *Portulaca oleracea*) and new Zeeland spinach (both leaf-crops), beetroot, and rapeseed. The later has been used extensively in The Netherlands during the start of land reclamation in the former sea-soils after the reclamation of Flevoland in the 1950's.

Cereal crops actually perform better, being naturally moderately tolerant with thresholds starting at 6 dS/m (with maize as an exception). An interesting candidate could be Sorghum (*Sorghum bicolor)* one of the main staple foods cultivated in Asia (the fifth most economically imported cereal crop worldwide) and rising trend in America and Western Europe being dubbed "the next quinoa" by Mintel foods trends 2017. It has a salt tolerance of around 6.8 ds/m but literature shows the presence of extensive genotypic variation (Tigabu *et al*., 2013). Wheat is listed in literature as comparable in salt tolerance, but field tests in The Netherlands with some wheat cultivars in the past (unpublished data Zilt Perspectief) have shown the crop to perform poorer than expected.

Legumes like beans, are high protein and contain many essential amino acids making them an excellent meat substitute and important in our saline research goal. However they often struggle to grow at a salinity level higher than 6 ds/m. One of the main theories why legumes struggle with the higher salt concentrations is that the nitrogen-fixing symbiotic bacteria in root nodules that are needed to sustain the plant, thrive poorly in higher saline conditions or are not present in the soil in adequate numbers.

Table 5. long list and in bold the short list of threshold salinity level (ECe = EC saturated paste) for field crops. For crops with several publications an average was taken to enable selection in our project. Reference numbers: 1: Manaa et al., 2019; 2: Diaz et al., 2018; 3: Stuyt et al., 2016; 4: Asci, 2011; 5: Ghaderi-Far et al., 2010; 6: van Dam et al., 2007; 7: Ghulam Muhammad et al., 2006; 8: Tanji & Kielen, 2002; 9: Shannon & Grieve, 1999; 10: Rogers et al., 1997; 11: Francois et al., 1990; 12: Shaddad et al., 1990.

Crops		Threshold (dS/m) EC saturated paste	Referenc (nb.)	Crops		Threshold (dS/m) EC saturated paste	Referenc (nb.)
Arable non N-fixing				**N-fixing species / legume**			
rapeseed	**Brassica napus**	**11.0**	**6**	**honey clover**	**Melilotus officinalis**	**8.0**	**5**
fodder beet	**Beta vulgaris**	**7.0**	**6**	**lupin**	**Lupinus angustifolius / termis**	**6.0**	**12**
sugar beet	**Beta vulgaris**	**6.4**	**3,6**	white clover	Trifolium repens	4.0	10
pursley	**Portulaca oleracea**	**6.3**	**9**	brown bean	Phaseolus vulgaris	3.5	6
Radish	Raphanus sativus	4.7	3,6	red clover	Trifolium pratense	3.3	4,8
andive	Cichorium endivia	4.3	3,6	alfalfa (lucerne)	Medicago sativa	2.0	2,8
asparagus	Asparagus officinalis	4.1	6	green bean	Phaseolus vulgaris	1.3	3,6
leek	Allium ampeloprasum	4.0	3	**cereals**			
tomato	Lycopersicon esculentum	3.5	3,6	**rye**	**Secale cereale**	**11.4**	**8**
chicory	Cichorium intybus	3.2	3,6	**barley**	**Hordeum vulgare**	**7.6**	**3,6,8**
cauliflower	Brassica oleracea	3.2	6	**winter wheat**	**Triticum aestivum**	**7.0**	**3,6,8**
red cabbage	Brassica oleracea	3.2	6	**spring wheat**	**Triticum aestivum**	**7.0**	**3,6,8**
white cabbage	Brassica oleracea	3.2	6	**sorghum**	**Sorghum bicolor**	**6.8**	**8**
brussels sprout	Brassica oleracea	3.2	3,6	**millet**	**Pennisetum americanum**	**6.5**	**7**
sweet pepper	Capsicum annuum	2.9	3,6	maize	Zea mays	2.0	3,6,8
cucumber	Cucumis sativus	2.8	3,6	**others**			
seedpotato	Solanum tuberosum	2.7	3,6	**quinoa**	**Chenopodium quinoa**	**>10.0**	**1**
potato	Solanum tuberosum	2.7	3,5	**caraway**	**Carum carvi**	**10.0**	**9**
lettuce	Lactuca serriola	2.5	3,5	**guar**	**Cyamopsis tetragonoloba**	**8.8**	**8**
carrots	Daucus carota	2.5	3,6	**cotten**	**Gossypium hirsutum**	**7.7**	**8**
onions	Allium cepa	2.5	3,5	**Kenaf**	**Hibiscus cannabinus L**	**6.3**	**8,11**
spinage	Spinacia oleracea	1.8	3,6	date-palm	Phoenix dactylifera	4.0	8
flax	Linum usitatissimum	1.7	6				

4 A global consumer perspective: towards selection of the experimental crops

While food shortages are a major problem in developing countries, most developed countries struggle with obesity caused by an overconsumption of processed sugar, fat and meat (e.g. Ranganathan *et al.*, 2016). Our current levels of meat consumption are not sustainable in the future. In order to feed the growing world population and while stemming the obesity epidemic; a shift towards a more plant-based diet would be a major step forward. This plant based diet should be rich in plant proteins, fibres and slow carbohydrates. Cereals and legumes are rich in the above mentioned traits and considered staple foods which makes them very suited for our project.

For this reason, to come to a selection of crops used in this project we filtered with the following four discriminators:

- The crops should be at least moderately salt tolerant as indicated in literature: in bold: crops with salinity thresholds above 6 dS/m – in table 5.
- Crops should have potential as staple crops or important crops in future diets to feed the world.

- At least one of the crops should be a legume or N-fixing crop, because this is a key-element in organic crop rotations.
- The crops should be able to grow under Dutch field conditions, so sub(tropical) crops will not be selected.

Following these selection criteria, the crops used in our saline experiments were:

1. *Sorghum bicolour*, varies genotypes: Sorghum is one of the major cereal crops in the world, a potential staple food, and various genotypes are currently introduced in The Netherlands as a new crop. It has a higher salt tolerance then wheat and maize, and its salt tolerance is known to show a wide genotypical variety.
2. Andean Lupin (*Lupines mutabilis*), that is said to be able to grown in very extreme conditions and non-fertile soils, and is a high potential new protein crop for the future (Caligari *et al*., 2000).
3. Honey clover (*Melilotus officinalis, Melilotus siculus*): in experiments performed by Brunning (2015) *Melilotus officinalis* was one of the legumes with the highest potential of growing under saline conditions. Rogers *et al.* (2011) show that in Australia 'messina' (*Melilotus siculus*) has shown extremely high potential to grow under saline and waterlogged conditions there. In 2018 also *Medicago polymorpha*, another legume was included. Nichols *et al*. (2009) describe it to be germinating at highly saline conditions (up to 240 mM NaCl which is around 22 dS/m), whereas Cherifi *et al.* (2011) describes a severe decrease in germination at 50 mM NaCl which is around 5 dS/m).

5 Field experiments: experimental setup

5.1 Two years of field trails

In the Netherlands, one of the few experimental facilities to test salinity tolerance of field crops exist on the isle of Texel: the "Salt Farm Texel" (https://www.saltfarmtexel.com/salt-farm-texel). Its uniqueness is that it is located on an organic farm (originally one of the oldest biodynamic farms of the country) and that therefore organic farming practices and sustainability are regarded highly. It recently got international attention, with a salt tolerant potato variety (The Guardian, McVeigh, 2014) and has won several international awards (Climate Adaptation business Challenge, USAID award) for its innovative approach. In this project we cooperated with the Salt Farm Texel, because it offered the opportunity of using their high-technology testing site with 7 levels of saline conditions in four replicates. The testing grounds are located on a sandy soil, but encompasses grounds with heavier soil types as well.

5.2 Fertilizer applications

In both years, a base dose of nutrients was applied in the form of compost and manure (both certified organic). The amount of compost added was 16 tons per hectare in 2017 (with 5.5 kg N/ton, 2.4 kg P_2O_5/ton and 3.4 kg K_2O/ton), and 12 tons/ha of manure (with 7.7 kg N/ton, 9.1 kg P_2O_5/ton and 6.5 kg K_2O/ton). In 2018, 20 ton/ha of compost was applied (with 5.6 kg N/ton, 1.7 kg P_2O_5/ton and 1.7 kg K_2O/ton) and also 12 tons/ha of manure (with 3.7 kg N/ton, 1.4 kg P_2O_5/ton and 6.0 kg K_2O/ton). In 2017 for the sorghum, and in 2018 for all crops, additionally an application of organic pellets was made. In 2017 this was 3.7 ton/ha of organic pellets (with N:P:K percentage of 4.0:3.0:3.0), and in 2018 this was 2 ton/ha (with N:P:K percentage of 3.2:0.5:1.4)

5.3 Salinity treatments and soil types

In this first year (2017) crops were tested on various levels of salt stress, but only on one soil type (sand). In the second year (2018) the tests were repeated, but also one of the saline levels (8 dS/m) has been laid out on a heavier soil type (clay-loam) as a comparison (clay particles (<2um): 15%). Measurements in the field included plant numbers, plaint height, weight, yield and quality aspects of the harvested material.

Crops were tested on 6 different salinity levels in the irrigation water, being 1, 4, 8, 12, 16 and 20 dS/m in both years. All salinity levels were applied in 4 replicates in a complete randomized design. To check on the effect of the saline irrigation water on the soil salinity, measurements have been made by salt farm Texel during the growing season at a frequency of 7 (2017) and 9 (2018) times in that season, respectively. These measurements were made using suction cups at two depths (0-10 cm and 20-30 cm in 2017; 5-15 cm and 25-35 cm in 2018, respectively), of which the average was taken. Soil samples of 0-30 cm were also taken (12 in 2017, 14 in 2018) of plot differing in salinity, in 10 subsamples per plot. ECe was measured using the international standard method, and the resulting correlation between EC pore water and ECe (R^2-value 0.92 in 2017 and 0.88 in 2018) was used to calculate ECe's on all dates that pore water measurements were made. These were averaged over the growing season and are shown in Table 6. Here we see that in general, variation in salinity levels was not great. However, in 2017 we see that the soil salinity level systematically has been somewhat lower than that of the irrigation water (that was controlled by a computerized regulation system), whereas in 2018 the soil salinity level was systematically a little bit higher that the irrigation water.

Tabel 6. Salinity of the irrigation water (target, as regulated by a computerized system) and actual salinity of the soil (ECe calculated from pore water measurements) at 7 (2017) and 9 (2018) measurement times during the growing season.

EC irrigation water	2017 ECe soil	2017 stdev	2018 ECe soil	2018 stdev
1	0.7	0.1	1.9	0.5
4	3.6	0.7	6.1	0.7
8	6.9	0.6	9.6	0.1
12	9.5	0.5	13.3	0.7
16	12.1	0.3	18.5	0.7
20	17.0	5.0	21.5	1.0

6 Experimental results

6.1 Sorghum

6.1.1 The cultivars

Sorghum is currently under development as a new field crop for Dutch conditions. For this purpose, a number of cultivars are being selected and tested in the Dutch climate by the "Hoeve Dierkensteen", Maatschap de Milliano-Meijer, https://www.hoevedierkensteen.nl/). The three cultivars used in this study were obtained from Hoeve Dierkensteen. All three cultivars are suited for human consumption (seeds), but also whole plants can be used as animal fodder. In both years, we used these three cultivars (Table 7). The exception is cultivar HD19-1W, of which the 7th generation was used in 2017, but the 8th generation (so one selection step further) in 2018. One of the cultivars, HD-CN1-1BL is *S. nigricans*, whereas the other two are both *S. bicolor*.

Table 7. Sorghum cultivars used in the field experiments.

2017	2018	type
HD-CN1-1BL	HD-CN1-1BL	*S. nigricans*
HD7-1C	HD7-1C	*S. bicolor*, coloured seeds
HD19-1W (7th gen.)	HD19-1W (8th gen.)	*S. bicolor*, white

6.1.2 The relative and absolute biomass as affected by salinity

Figure 2 shows the relative biomass plotted against the EC in the soil in 2017 and 2018, for the three sorghum cultivars. Dotted lines indicate 4 and 8 dS/m, the upper borders for slightly and moderate saline soils (Ivushkin *et al.*, 2019; FAO). We see that for the EC's below 10 dS/m, the sorghum crops performed relatively better in 2018 than in 2017. Yields at 4 dS/m were 40% to 60% that of yield in non-saline conditions in 2017, and 70% to 85% that of yield in on-saline conditions in 2018. The effects of salinity seemed less severe in 2018. Also, there were clear differences between the three cultivars: in both years, at the EC's below 10 dS/m we see that the cultivar HD-CN1-1BL was relatively less affected by salt than the other two cultivars. At EC's above 10 dS/m, in 2017 this was also the case, whereas in 2018 cultivar HD7-1C was relatively the least affected.

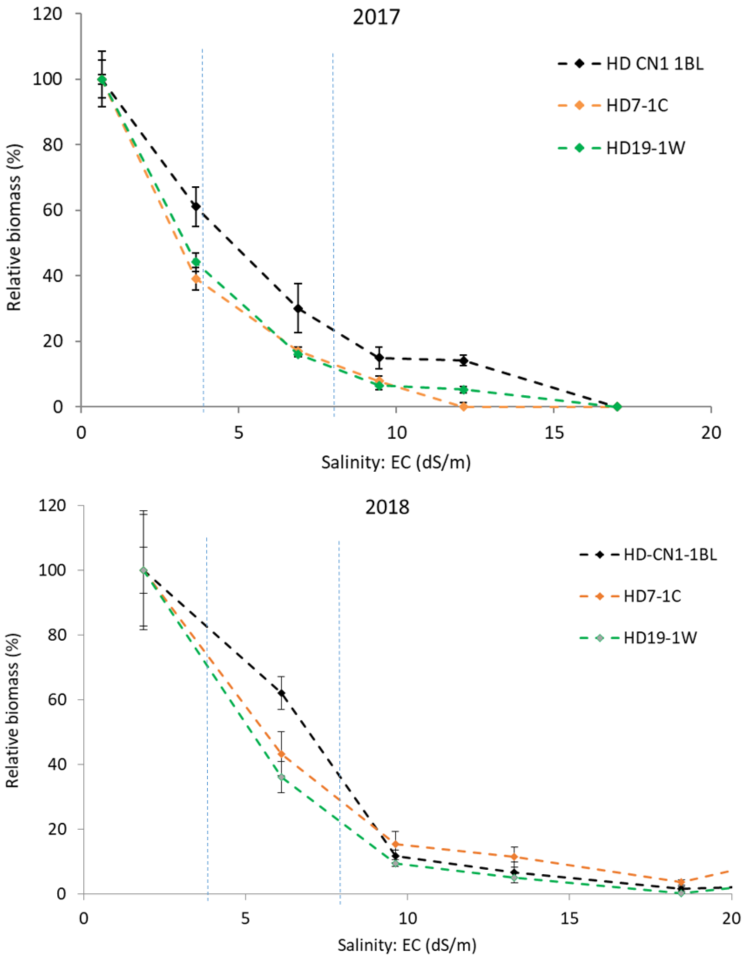

Figure 2. Relative biomass yields of the three sorghum cultivars grown under saline conditions in 2017 and 2018. Dotted lines indicate 4 dS/m and 8 dS/m, respectively.

Figure 3 shows absolute yields of dry matter of the three sorghum cultivars in our experiments. Here we see that the biomass in general was much higher in 2018 than in 2017, due to a difference in growing conditions (a very warm summer in 2018). At the upper level of salinity of slightly saline soils (4 dS/m) we see dry matters yields of 2 to 3 ton/ha in 2017, and of 4 to 9 ton/ha in 2018. At the upper level of salinity of moderately saline soils (8 dS/m) we see low yields, just under 1 ton/ha in 2017 and 1 to 3.5 ton/ha in 2018. In 2018, the year with the warmer growing conditions and higher productivity of Sorghum, we see that the cultivar that performed best in absolute sense was HD7-1C. This cultivar was effected more strongly by salinity than HD-CN1-1BL, but its productivity was so high that it was still the heaviest crop at higher levels of salinity. This points out two strategies in terms of finding crops for saline soils:

- firstly, one can look for crops and cultivars that are less effected and more salt tolerant
- secondly, one can look for absolute high productivity. Such crops can still have reasonable productivity under saline conditions if not effected in a much greater degree than others.

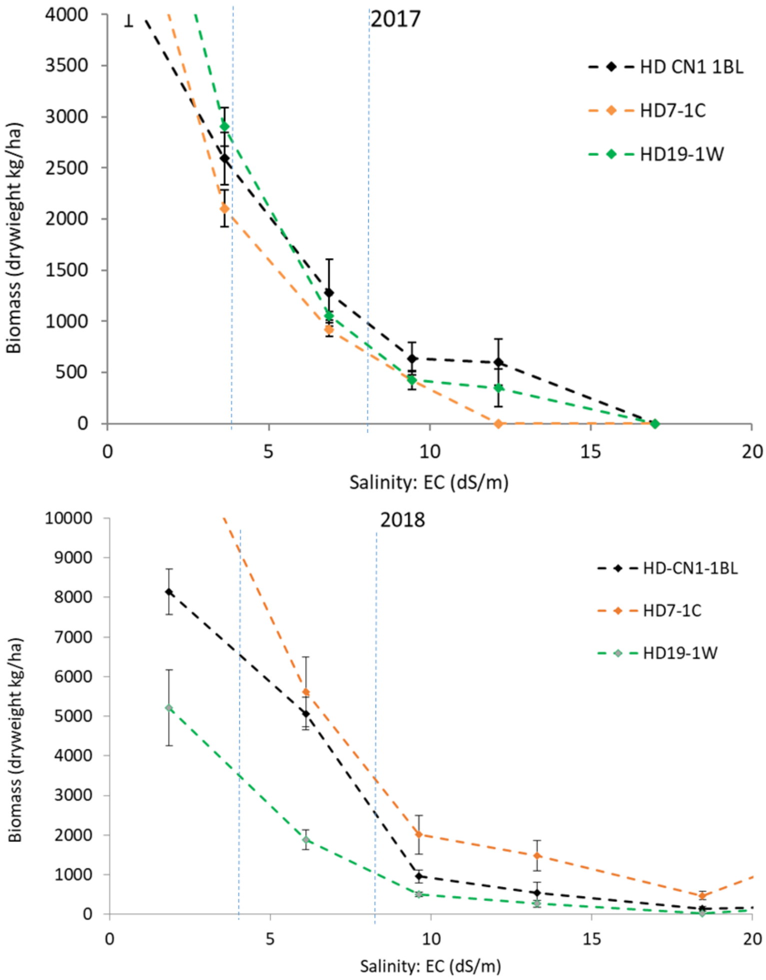

Figure 3. Dry matter biomass yields of the three sorghum cultivars grown under saline conditions in 2017 and 2018. Dotted lines indicate 4 dS/m and 8 dS/m, respectively.

6.1.3 The weight of the floral structures as affected by salinity

Figure 4 shows the relative weight of the floral structures, the seed weight, as effected by salinity treatment. Here we see that seed weight in general is less effected by salinity treatment than the whole plants. At the upper level of salinity of slightly saline soils (4 dS/m) we see that the floral structures had 40% to 60% of the weight of those in non-saline conditions in 2017, and 70% to 100% in 2018. At the upper level of salinity of moderately saline soils (8 dS/m) we see low yields, this was 20% to 30% of the seed weight at non-saline conditions in 2017, and 30% to 50% in 2018.

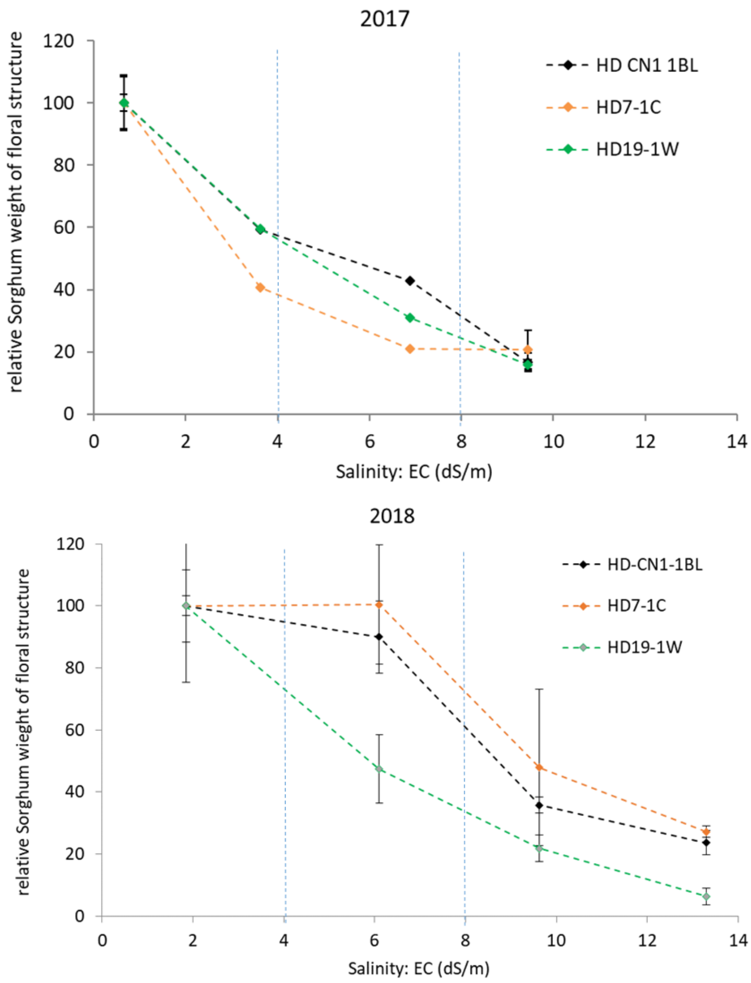

Figure 4. Relative weigh of the floral structures of the harvested sorghum, plotted against salinity treatment.

6.1.4 Sorghum yield on clay-loam under saline conditions

In 2018, the three Sorghum cultivars were grown also in a clay-loam field with saline irrigation (8 dS/m) during the growing season (Table 8). Although all other management has been comparable to that of the Sorghum grown in the sandy soil, biomass yield of the sorghum cultivars on the more heavy soil was much greater. It ranged from 16.6 ton/ha of dry matter (cultivar HD19-1W, the cultivar that also had the lowest yield on sandy soil) up to more than 24 ton/ha (cultivar HD7-1C, the cultivar that also had the highest yield on sandy soil in that year). The yields at the salinity level of 8 dS/m in this soil, (which is not a sandy soil any more but also not a heavy clay soil) were even higher that the yields at the lowest salinity level on sandy soil.

Table 8. Dry matter of the three Sorghum cultivars on a more heavy soil (clay-loam, 15% clay) compared to the dry matter on Sandy soil in the same year.

2018, Sand versus Clay-loam	**Biomass (dryweight kg/ha)**			
Cultivar	**Clay-loam, 8 dS/m**	Sand, 1.9 dS/m	Sand, 6.1 dS/m	Sand, 9.6 dS/m
HD-CN1-1BL	**24276**	8144	5611	953
HD7-1C	**17505**	12945	5064	2013
HD19-1W	**16604**	5216	1886	499

6.1.5 Mineral contents and quality aspects of Sorghum grown under saline conditions

Table 9 represents measurements of the quality of Sorghum at low level of soil salinity, and their changes under saline conditions (salinity level 9.5 (2017) and 9.6 (2018) dS/m). Significant positive effects of the salt that occurred both years were: an increase in digestibility (or i.o.w. easily digestible organic matter) of 8% to 13%, increase in magnesium (15%-18%) and a great increase in iron content (56%-120%). Furthermore, we measured mostly an increase in sodium but not for all cultivars, and mostly an increase in nitrogen content, but also not for all cultivars. Significant negative effects of the salt that occurred both years were a decrease in phosphorus content (-29% - -58%) and a decrease in copper content (-51% - -69%).

Table 9. Effects of saline conditions on mineral contents and quality aspects of Sorghum biomass. Statistical significant differences (p<0.05) are indicated with green (increase), blue (decrease) or both (grey). "Int" indicates different reaction for different cultivars. In the most right column "Y" indicates if the significant effect occurred both years.

Sorghum quality	Value at lowest salinity (average: 1.3 dS/m), average over years and cv's	2017			2018			Effect both years
		P-value	sign	effect size	P-value	sign	effect size	
Digestability of the organic matter (Tilly & Terry) (% OM)	62.9	<.001	plus	+13%	0.003	plus	+8%	Y
Sugar (g/kg)	69.4	not analysed			0.053	min	-26%	
Starch (g/kg)	68.3				0.003	min	-70%	
Na (g/kg)	3.2	0.009	plus, int	+25% - +55%	0.002	plus or min	-15% - +60%	Y*
K (g/kg)	16.3	0.001	min	-14%				
Mg (g/kg)	2.9	0.006	plus	+18%	0.007	plus	+15%	Y
Ca (g/kg)	2.7							
P (g/kg)	3.8	<.001	min	-36%	<.001	min, int	-29% - -58%	Y
S (g/kg)	1.4	0.007		+13%				
Mn (mg/kg)	15.8							
Zn (mg/kg)	43.6							
Fe (mg/kg)	179.2	<.001	plus	+120%	0.008	plus	+56%	Y
Cu (mg/kg)	5.8	<.001	min	-56%	<.001	min, int	-51% - -69%	Y
N-total (g/kg)	12.4	<.001	plus	+43%	0.004	plus or min	-24% - +22%	Y*

The increase in magnesium content as reported above seems interesting in terms of human nutrition: a too low intake of magnesium has been a concern for a long time (Lui & Lui, 2018; Vormann, 2003; Ford & Mokdad, 2003). Views on whether our western diets provide for enough magesium differ, as do levels extra and intracellular levels of magnesium differ a lot between individuals. Recommended intake of magnesium is between 300 and 420 mg/day (Vormann, 2003) , and mean intake in a sample of the U.S. population in 1999 and 2000 was around 290 mg/day. If we look at the Sorghum measurements, the contents of magnesium were 2900 mg/kg dry matter. An increase of 15% to 18% by effects of salinity would be and extra 435mg to 522mg per kg consumed material. It seems that such an effect would be interesting, purely in terms of size.

Iron deficiency is one of the leading risk factors for disability and death worldwide (Zimmermann & Hurrell, 2007). As described by these authors, risk on iron deficiency is especially pronounced in plant based diets, as the uptake of iron in plant material is lower. Off all iron present in plant based diets, only 5%-10% is available depending on the level of vitamin C. Recommended daily intakes, taking this low uptake factor in account (so for diets low in animal

protein), range from 13.7mg up to 27.4mg for adult men, and from 29.4mg up to 58.8mg for adult women worldwide (Zimmermann & Hurrell, 2007). In Sorghum we find 179.2mg Fe per kg dry biomass. Effects of salinity were increases of 56% to 120%. This results in 100mg to 215mg extra iron per kg consumed material. Again, purely in terms of size of the effect, this would be very interesting if found in a wider range of products.
Andean Lupin (*Lupinus mutabilis*)

6.2 *Lupinus mutabilis* (Andean Lupin)

6.2.1 The cultivars used

For Andean Lupin, in 2017 a first field experiment was done using only one line (it was still quite diverse and wild) was used, LIB223(synonym 'Choco Beans'). In the second year, LIB223 plus three other lines were used (Table 10).. All *lupinus mutabilis* lines are eventually intended to be used for human consumption, as a protein crop. In 2017, all cultivars were transplanted at around 10 cm height at 21-6. In 2018, cultivars were sown from inoculated seeds at 18-5. Table 10 gives a short overview.

Table 10. Cultivars Lupinus mutabilis used.

	2017	2018
1	LIB223	LIB223
2		LIB221
3		LIB220
4		LIB222

6.2.2 Number of surviving plants during the season

Figure 5 shows the number of surviving plants in the saline plots. In 2017 we see that *L. mutabilis* numbers quite rapidly decline during the growing season, at the higher levels of soil salinity. In 2018, a wider range of genotypes of *L. Mutabilis* were used. Here, we see a comparable decline in number of surviving plants during the growing season. LIB223, the cultivar used in 2017, was actually the most salt tolerant of the four genotypes used. The dying off of plants resulted in no or only a very few plants surviving untill the end of the growing season in the salinity levels higher than 4 dS/m (top level of slightly saline soils, Ivushkin *et al.*, 2019; FAO).

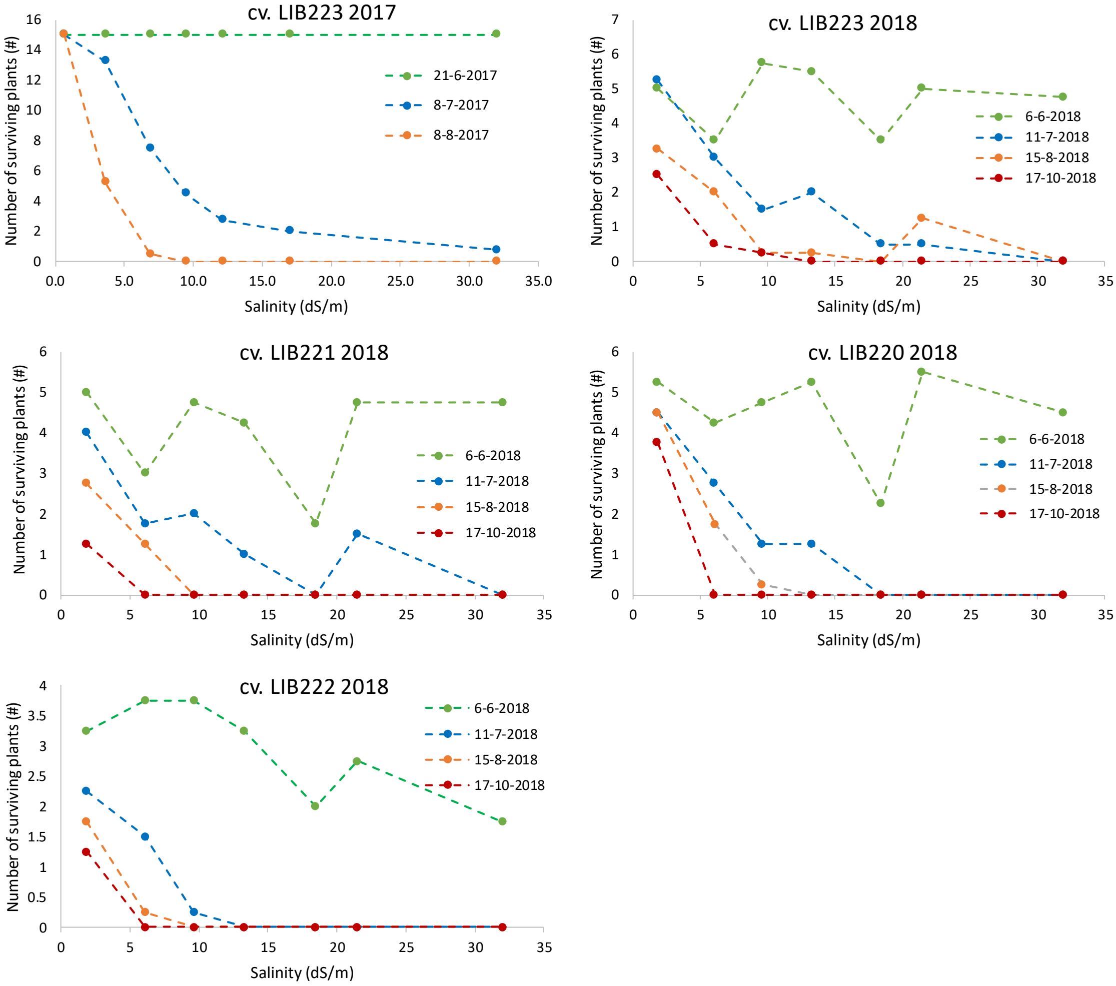

Figure 5. Number of surviving plants at different dates during the growing season, in 2017 and in 2018 for all cultivars.

6.2.3 Above ground fresh biomass at the end of the growing season

Figure 6 shows the above ground biomass, per planted *L. mutabilis*, at the end of the growing season, in 2017 and 2018. Again, the blue dotted lines indicate top levels of salinity of slightly saline soils (4dS/m) and moderately saline soils (8dS/m) (Ivushkin *et al.*, 2019; FAO). We see that in terms of biomass production, there was only some production at our lowest level of soil salinity (0.7 dS/m in 2017 and 1.85 dS/m in 2018). One salinity treatment higher in our experiments (3.6 dS/m in 2017 and 6.1 dS/m in 2018) resulted in very few surviving plants and hence a very small biomass. It shows that *L. mutabilis* genotypes that we used are surely not the salt tolerant protein crop species that can be grown in the highly saline conditions found in the north of The Netherlands. However, worldwide, the conclusion could be somewhat different: Ishushkin *et al.* (2019) also show that 97% of all saline soils worldwide, 1036.2 Mha as estimated in 2016, is saline but below 4 dS/m. We did only test one level of soil salinity below this threshold. To assess if *L. mutabilis* could still grow on part of this area, a test including more levels of slightly saline conditions would be useful.

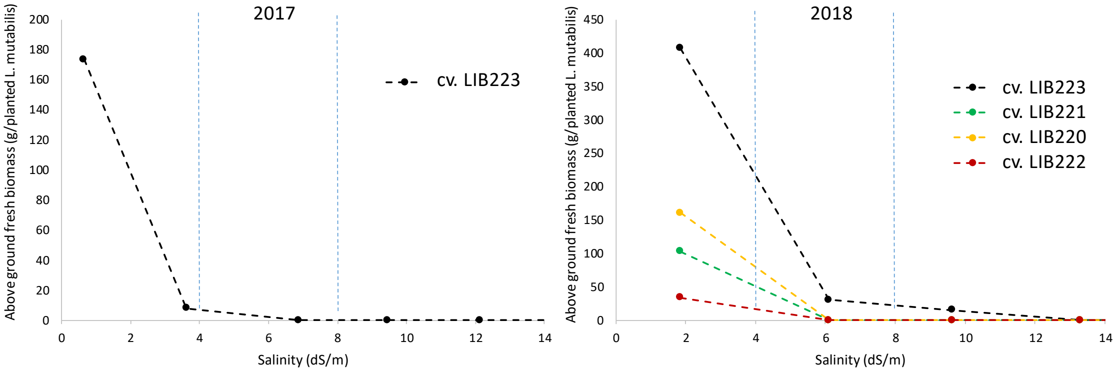

Figure 6. Above ground biomass of Lupinus mutabilis in 2017 and 2018, plotted against levels of soil salinity.

6.3 Green manures

6.3.1 The cultivars/species used and sowing dates

In 2017, experiments were performed with *Melilotus officinalis*, of which seeds were obtained that are for sale as a commercial green manure (https://www.vreeken.nl/391100-honing-klaver-citroengele-citroengeel). In 2018, some seeds of *Melilotus siculus* (messina) were imported from Australia and used in our experiments. Also included were seeds of *Medicago polymorpha*.

Due to the procedures necessary for importing, seeds were obtained quite late, and whereas *Melilotus officinalis* was sown on 6 june and 14 June (second sowing due to bad germination because of the drought) *Melilotus siculus* and *Medicago polymorpha* could only be sown at 27 June, 2-3 weeks later than *Melilotus officinalis*.

All used crops are green manures that fix nitrogen through symbioses. *Melilotus officinalis* has been used as a forage crop, but is (in parts of North America) a potential invasive weed, and can get toxic to livestock when infected by moulds (that convert a plant glycoside to coumarol and dicoumarol). *Melilotus siculus* is cultivated as a salt and water tolerant forage crop in Australia. *Medicago polymorpha* has been used as cover crop and green manure, and has shown varying results in salt tolerance during germination (Nichols *et al.*, 2009; Cherifi *et al.*, 2011). Table 11 gives a short overview.

Table 11. Cultivars of green manures used.

	2017	2018
1	*Melilotus officinalis*	*Melilotus officinalis*
2		*Melilotus siculus*
3		*Medicago polymorpha*

6.3.2 The relative and absolute biomass as affected by salinity

Figure 7 shows the above ground fresh biomass at the end of the season for the green manures, as affected by salinity. In 2017, we see that *Melilotus officinalis* shows a linear relation with salinity, from 18 ton/ha of fresh biomass at 0.7 dS/m decreasing to almost zero ton/ha at a salinity of 12 dS/m. At the upper threshold of slightly saline soils (4 dS/m), interpolation results in a fresh above ground biomass around 11 ton/ha, and at the upper level of moderately saline soils (8 dS/m) an above ground biomass of around 4 ton/ha. In 2018, we see that the biomass at the lowest salinity level was lower than in 2017, being around 12 ton/ha for *M. officinalis*. De decrease in biomass due to salinity was less. Linear interpolation resulted in around 11 ton/ha at 4 dS/m and still around 8 ton/ha at 8 dS/m. The difference between the two years might have been due to the extreme hot and dry conditions in the growing season in 2018. Higher growth rates at the low salinity level might not have been feasible for the crop due to these extreme field conditions.

Compared to *Melilotus officinalis*, we see much lower biomass for *Melilotus siculus* and *Medicago polymorpha*. This was caused by very late emergence, only in the beginning of August in 2018. *Melilotus officinalis* and *Medicago polymorpha* were sown already later the M. *oficinalis*, but then took over a month to germinate. The irrigation by the dripping system did not wet the first few centimetres of the topsoil, where the seeds were located. There was no rain from sowing of these crops until 28 and 31 of July in 2018, when around 10 and 4 mm fell, respectively. These two rain events lead to germination of the seeds in the beginning of August. For these two species the relative biomass is there for more interesting.

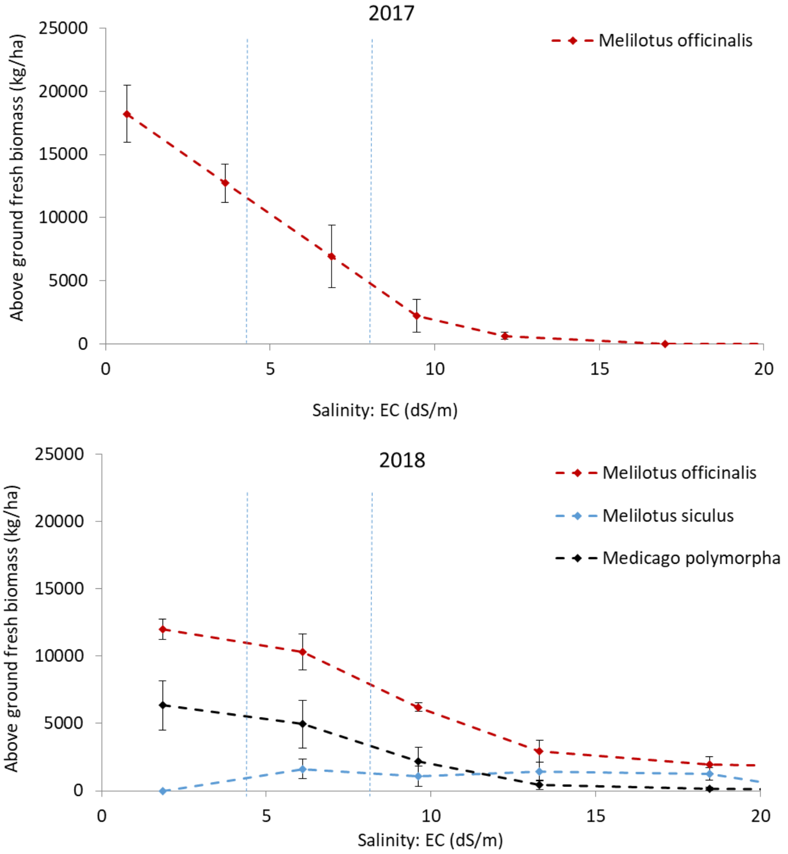

Figure 7. Above ground fresh biomass of the green manures, as affected by salinity in 2017 and 2018.

Figure 8 shows the relative biomass of the green manures as affected by salinity. We see again the linear decrease in biomass with salinity level for *M. officinalis* in 2017. This resulted in

60% and 30% of the biomass at 4dS/m and 8 dS/m, the upper levels of slightly saline soils and moderately saline soils, respectively. In 2018, we see that the effect of increasing salinity was smaller (probably to a suppressed growth rate at lower salinity level). This resulted in 90% and 70% of the biomass at 4 dS/m and 8 dS/m, respectively.

Compared to *Melilotus officinalis*, we see that *Medicago polymorpha* was effected a little bit more by increased salinity, resulting in 80% and 50% of the biomass at 4 dS/m and 8 dS/m, respectively.

Melilotus siculus had no plants at the lowest salinity treatment (no germination). Therefore, the relative biomass was calculated to the biomass at 6.1 dS/m. Overall growth rate and biomass for this species under our conditions was very low. Interestingly, the salinity of 6.1 dS/m up to 18.5 dS/m did not result in a decrease in biomass for this species.

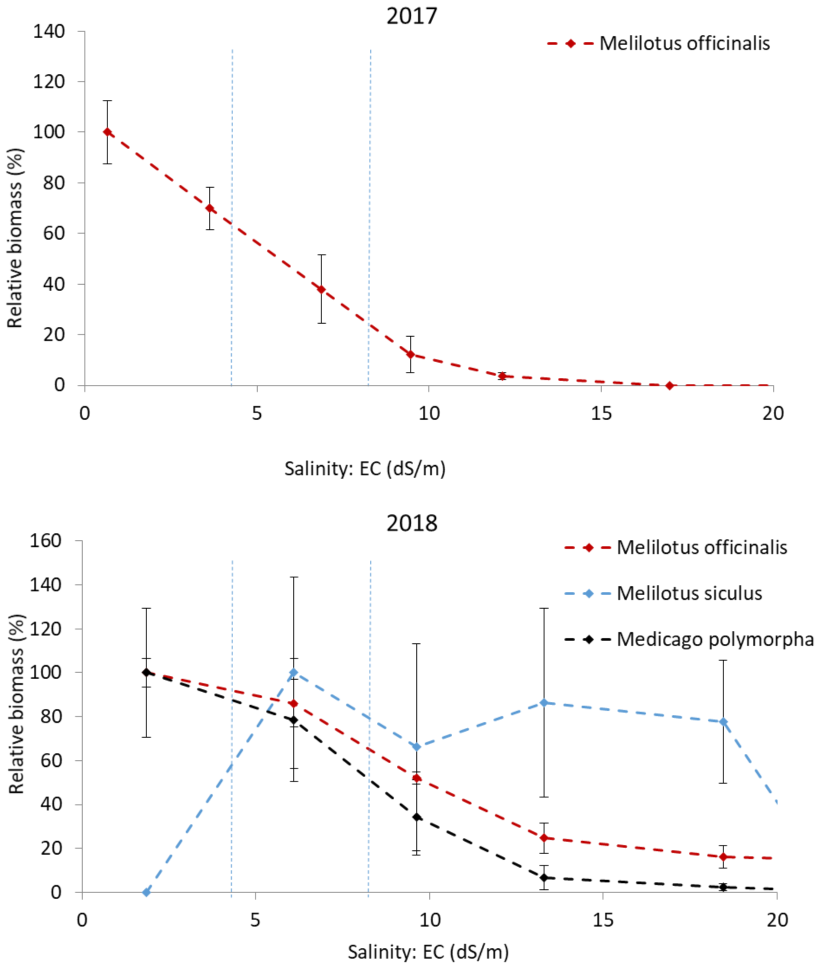

Figure 8. Relative above ground bioamss of the green manures, as affected by salinity in 2017 and 2018.

6.3.3 Green manures on clay-loam under saline conditions

Table 12 shows the performance of the green manures on heavier soil in 2018. On this soil type, we would expect less effects of the heat and drought, and we did see much more biomass for Sorghum. However, for the green manures, this is not the case: we see that there is a slight shift in performance. Although sown 2-3 weeks later, *Medicago polymorpha* had a higher growth rate on this soil type and at the end of the season had more above ground biomass that *Melilotus officinalis*. *Melilotus siculus* again had low biomass due to slow germination and growth under these conditions.

Table 12. Biomass of green manures under saline condition (8dS/m) on a clay-loam soil

2018, Sand versus Clay-loam	**Biomass (freshweight kg/ha)**			
Green manure	**Clay-loam, 8 dS/m**	Sand, 1.9 dS/m	Sand, 6.1 dS/m	Sand, 9.6 dS/m
Melilotus officinalis	**4160**	11970	10310	6210
Melilotus siculus	**560**	0	1620	1070
Medicago polymorpha	**6320**	6330	4960	2160

6.3.4 Nodulation and total nitrogen

To assess if any nodulation took place in the *Melilotus officinalis* crops grown under saline conditions, plants have been dug up and visual screening of the root systems has been done (Figure 9). Nodulation has been observed for all *Melilotus officinalis* plants that have been investigated, at 1.4 dS/m, at 13.7 dS/m and even at 17.5 dS/m. At least some nitrogen fixing seemed to occur at even high levels of salinity. At the higher levels of salinity, plants were much smaller. Although no quantitative assessment was made, the root system of these plants was smaller, and less nodules were found. Also, the colouring of the nodules at their insight seemed less bright (indicating lower activity).

Figure 9. Nodulation in plots with different levels of salinity in 2018, at plants of Melilotus officinalis.

Table 13 shows the above ground biomass and the total nitrogen (in kg/ha) in the *Melilotus officinalis* crops in 2017 and 2018 for the different salinity levels. We see that at 4 dS/m (the upper border of lightly saline soils) *Melilotus officinalis* crops can be expected to accumulate around 70 to 80 kg of N per ha in the above ground biomass. We know that at least part of this nitrogen has been fixed. These 70 to 80 kg of nitrogen seems not much. However, one should consider a saline crop rotation in such a saline soil. If a second crop in this rotation would be Sorghum as we tested in our research, the average dry matter biomass at this salinity levels would be around 5000 kg/ha, with an average nitrogen content of 12.4 g/kg. This means nitrogen content of the above ground biomass of this crop would be around 62 kg N/ha. Seen in this perspective, the 70 to 80 kg N/ha in the above ground biomass of the Melilotes crop can be quite substantial.

Table 13. Above ground fresh and dry biomass and N in Melilotus officinalis crops in 2017 and 2018.

Salinity	Fresh above ground biomass	Above ground dry matter	N in above ground biomass
		2017	
dS/m	kg/ha	kg/ha	kg/ha
0.7	18213	4061	93
3.6	12725	2786	72
6.9	6913	1476	44
9.5	2213	460	15
12.1	638	129	5
17.0	0	0	0
32.0	0	0	0
		2018	
Salinity	Fresh above ground biomass kg/ha	Above ground dry matter kg/ha	N in above ground biomass kg/ha
1.9	11970	4714	95
6.1	10310	4060	82
9.6	6210	2446	49
13.3	2950	1162	23
18.5	1920	756	15
21.5	1810	713	14
32.0	40	16	0

7 Conclusions and recommendations

7.1 Hypothesis 1: saline crops are promising additives within the plant based diets

From this study, we can conclude that salinity will change the type of crops as we currently know in our region. Only relatively few crops are able to grow in saline conditions, and for saline agriculture crop rotations will therefore consist of other crops, and probably also crops from other regions of the world. Partly this will also include new crops: plant species that could be used, have relative high tolerance to salinity, but are not well developed crops yet. A wider variation of plant based products resulting from such rotations on itself would be a valuable addition towards more sustainable and/or plant-based diets.

Secondly, we conclude that there are significant changes in the mineral composition within saline crops. In Sorghum biomass, we have measured increases in iron and magnesium contents in both experimental years, that were large enough to be interesting for human nutrition. We recommend further research that investigates if these/such changes happen in a wider range of crops, and to what extent. They could be used as valuable assets in the sustainable diets of the future.

7.2 Hypothesis 2: saline soil conditions require adapted crop rotations based on salt adapted crop species to be truly sustainable for these areas

Firstly, it is very important to realize that salinity lowers yield: even at relatively low levels and even for tolerant crops, yields that we realized in this research under saline conditions (and also in all previous research under saline conditions) were substantially lowered. The only exception may be halophyte species. These are, at least for our conditions, still mostly very undeveloped and focused on niche markets (if grown at all).

As stated above, only relatively few crops are tolerant to high salinity levels. However, most of the saline soils worldwide (97%) have only levels of salinity up to 4 dS/m, and many crops have threshold EC levels that are somewhere between 1 and 4 dS/m. This means our hypothesis holds for higher salinity levels, but not for all slightly saline soils.

We recommend further development of real halophytes as crops. Furthermore, we recommend research that specifically focusses on salinity levels of 0 up to 4 dS/m.

7.3 Hypothesis 3: salt tolerant green manure cultivars can be selected to enable organic saline rotations

In this research we have shown that salt tolerant green manures can be found, and that at least some nitrogen fixing took place at higher salinity levels. *Melilotus officinalis* seems to hold good perspectives for our Dutch climate, especially on sandy soils. Although it had a slow growth rate we cannot fully discard *Melilotus siculus* yet, because we had an extreme year (very dry conditions after sowing) and an short growing period. *Medicago polymorpha* performed relatively well on clay-loam, and not so well on sandy soil.

7.4 Hypothesis 4: saline conditions require adapted crop management to enable sustainable production and long term soil fertility

Concerning crop management, it needs to be adapted to lower yields of saline conditions. This would imply a more extensive management strategy to enable sufficient financial returns, and adapted fertilization to prevent losses. Furthermore, implementing green manures in organic rotations would often imply a whole season with a green manure crop. Having relatively low nitrogen yields, a stronger dependency on manure input can be expected. While stating this hypothesis in our original project proposal however, we were thinking of long term soil structural and quality problems due to saline conditions. These, we have not seen in our experiments. We have to state in this context that most of our experiments have been performed on sandy soils. We had a small second experimental plot on a clay-loam soil, but the general growing conditions were quite good. Since farmers in practice seem to experience severe soil quality problems, we recommend more elaborate experiments on more and more heavy soils to investigate this aspect.

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