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Author for correspondence:
Bhaniswor Pokhrel, E-mail: bhaniswor.pokhrel@food.au.dk

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Processing methods of organic liquid fertilizers affect nutrient availability and yield of greenhouse grown parsley

Bhaniswor Pokhrel[1], Jorn Nygaard Sorensen[1], Henrik Bjarne Moller[2] and Karen Koefoed Petersen[1]

[1]Department of Food Science, Aarhus University, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark and [2]Department of Engineering, Aarhus University, Hangovej 2, DK-8200 Aarhus N, Denmark

Abstract

The demand for organic foods is increasing globally, but a key limiting factor to the production of organic greenhouse produce is the lack of certified liquid fertilizers. In this experiment, four organic fertilizers were produced using either acidic extraction, anaerobic digestion or both of ensiled biomass of organic red clover and white mustard. The resulting fertilizers were applied to greenhouse-grown parsley either alone, or in combination with nitrogen (N)-enriched water produced by flushing acidic water with ammonia, to determine their effect on plant growth and the nutrient concentrations of parsley. Six combinations of fertilizer treatments were included in the greenhouse experiment. Three treatments received either fertilizers derived from acidic extraction, anaerobic digestion or both and three treatments received fertilizers derived from acidic extraction combined with N-enriched water. Conventional inorganic liquid fertilizer, chicken manure extract and no liquid fertilizer (only water) were added as control treatments. A higher N-min (ammonium and nitrate) to potassium (K) ratio was found in fertilizers after anaerobic digestion compared to acidic extraction. All organic fertigation treatments resulted in high pH, high K and chloride concentrations and high NH_4/NO_3 ratios in the root zone. In addition, high electrical conductivity (EC), P, K and Mg concentrations were found when only acidic extracted fertilizers were applied. Application of plant-based organic fertilizers without amending with N-enriched water resulted in biomass yields that were 21–26% lower than the inorganic fertigation control. However, fertigation with chicken manure extract, or a combination of fertilizer derived from acidic extraction and N-enriched water, resulted in similar plant growth as inorganic fertilizer. The lower yield from fertilizer derived from acidic extraction was due to elevated EC levels in the growing medium. Our results suggest that yield of greenhouse-grown parsley using either organic fertilizers combined with N-enriched water or chicken manure extract is similar to conventional fertilizer.

Introduction

Production and consumption of organic foods are seeing double-digit annual increases on the global market as well as the Danish market (IFOAM, 2015; Statistics Denmark, 2016). Worldwide sales of organic food and drink reached 80 billion USD in 2014, which was an increase of 16.8% compared with 2013. Organic food accounts for about 8% of the total food market in Denmark (Statistics Denmark, 2016), which is eight times higher than the world average. Sales of organic fruits and vegetables in Denmark increased by 28% between 2014 and 2015 and now make up 26% of the organic food market (Statistics Denmark, 2016). This increasing demand for organic products provides a great opportunity for Danish organic vegetable growers to increase their production. However, productivity in organic farming is often lower than in conventional systems. A key yield-limiting factor in organic production systems is the lack of suitable organic fertilizers that can be approved and certified according to Danish rules on organic greenhouse production. In Denmark, Sweden, Finland, South and North America including Canada, organic greenhouse production in demarcated beds is allowed and liquid fertilizer is used to supplement solid fertilizer incorporated in or placed on top of the growing medium. One challenge is the variability in nutrient content and complexity of the raw materials used for organic fertilizers (Treadwell et al., 2007).

Organic greenhouse producers often incorporate solid organic fertilizer into the growing medium prior to planting and supplement with liquid and/or solid fertilizers (Burnett et al., 2016). For example, the use of a peat-based substrate mixed with 50% compost and combined with organic liquid fertilizers gave the highest yield in a tomato crop and similar to that obtained in a conventional system (Zhai et al., 2009). Therefore, liquid fertilizers applied through fertigation can provide the nutrients required for satisfactory crop growth during the different developmental stages of plant growth. Liquid fertilizers for greenhouse herbs can be applied through drip-feed or flood-and-drain irrigation (fertigation). Many organic liquid fertilizers are available globally, but only a few of these are certified for use in Denmark. Organic liquid fertilizers can be produced locally by methods such as extraction, fermentation and anaerobic digestion of plant- and animal-based biomass (Succop and Newman, 2004; Martínez-Alcántara et al., 2016). The concentration and composition of nutrients and their availability in an organic liquid fertilizer depend on the type of biomass and the production method used (Hartz et al., 2010; Martínez-Alcántara et al., 2016). Available nitrogen (N) in the liquid fraction of anaerobically digested chicken manure can be about 60–80% of total N (Bujoczek et al., 2000), however, can be as low as 46–53% of total N in water extract (Gross et al., 2008). Nutrients can be extracted more efficiently from manure when using alkaline extraction rather than pure water (Tortosa et al., 2014).

The yield and quality of crops given organic liquid fertigation depend on the nutrient composition and concentration in the fertilizer, crop species, type of growing medium used and root zone conditions (Succop and Newman, 2004; Pokhrel et al., 2015). Balancing the supply of essential nutrients from plant-based liquid fertilizers to meet crop demand is a challenge because the concentrations of potassium (K), chloride (Cl) and sodium (Na) in plant-based liquid fertilizers are often excessive compared with N (Pokhrel et al., 2015; Martínez-Alcántara et al., 2016). An optimal supply of N for crop growth, therefore, creates suboptimal growing conditions in the root zone because of low N : K and N : Cl ratios and high K : Ca ratio and electrical conductivity (EC) (Martínez-Alcántara et al., 2016; Pokhrel et al., 2017a; b). In demarcated beds and pots, chemical properties (pH, EC and nutrient concentrations) can change quickly as the growing medium is often peat based and the volume of the growing medium restricted. Therefore, following growing medium pH, EC and nutrient concentrations at regular intervals is important to adjust and avoid unfavorable root zone conditions. The unfavorable conditions could be overcome by combining plant-based fertilizers with N-rich fertilizers, which will reduce K and Cl concentrations in the fertigation solution and thereby reduce EC and create more favorable root zone conditions for plant growth.

In this experiment, liquid organic fertilizers were produced from red clover and white mustard silage using either acidic extraction, anaerobic digestion or both. Red clover was included as N-fixing crop and white mustard because it is a common, fast-growing catch crop in Denmark. Anaerobic digestion is commonly used for waste recycling and bioenergy production and the liquid fraction has potential as a high-value by-product for crop nutrition (Bujoczek et al., 2000). Acidic extraction of plant biomass for production of liquid fertilizer requires less time compared with anaerobic digestion and is currently used by one Danish company (GreenF Aps, Denmark).

In this study, we hypothesized that: (1) the nutrient concentration and composition of liquid organic fertilizers are influenced by the type of raw material and extraction method used, and (2) supplementing plant-based fertilizers with N-enriched water improves the nutrient composition and EC in the growing medium and thereby increases yield. To test these hypotheses, we applied liquid fertilizers derived from acidic extraction, anaerobic digestion or both of red clover and white mustard either alone or in combination with N-enriched water (produced by flushing acidic water with ammonia) to greenhouse-grown parsley for 21 days. Growing medium pH, EC and nutrient concentrations, biomass yield parameters and shoot mineral nutrient concentrations were quantified.

Materials and methods

Plant materials and growing conditions

A greenhouse study was conducted between March and April, 2015, at the Department of Food Science, Aarhus University, Denmark. A growing medium of blond peat moss (0–20 mm with 2.7 kg m^{-3} limestones, 40 kg m^{-3} granulated clay (2–6 mm) and pH 6.0; Pindstrup Mosebrug A/S, Denmark) was used for all treatments. Parsley was used as an experimental crop because yield and quality quickly respond to nutrient deficiency and it has a short growth cycle. Twelve seeds of parsley (*Petroselinum crispum* Mill. cv. Felicia, Rijk Zwaan, the Netherlands) were sown directly in 11 cm diameter pots. Pots were kept in a climate-controlled room and subjected to an 18-h photoperiod of 150 µmol m^{-2} s^{-1} artificial light (Green Power LED deep red & white, Philips, Poland) and a 20°C day and night temperature to secure a good germination rate. Prior to transferring plants to the greenhouse for fertigation treatments the number of plants in each pot was adjusted to 8 by removing excess plantlets. The greenhouse temperature set point was 18°C and roof vents were opened when the temperature went beyond 21°C. Plants were grown at 18 h photoperiod (6–24) and supplementary lighting was switched on or off when the outdoor solar intensity was below 110 or above 150 µmol m^{-2} s^{-1}, respectively. The greenhouse screen was closed during sunny periods when the outdoor light intensity was above 1200 µmol m^{-2} s^{-1}. Plants were fed nutrient solutions through flood-and-drain fertigation starting 19 days after sowing, and the drainage water was recirculated to individual fertilizer tanks for each treatment. Acid-extracted fertilizers, anaerobically digested fertilizers and N-enriched water were prepared prior to the experiment. Once a week fertigation solutions were prepared from these stock solutions as well as chicken manure extract from composted chicken manure and used to replace fertigation solutions in individual fertilizer tanks. All fertigation solutions were replaced once a week with fresh once prepared from stock solutions except chicken manure extract, which was freshly prepared each week. Plants were fertigated according to need, which was every 2 days at the beginning and three times daily at the end of the experiment.

Production of organic liquid fertilizer stocks

Four plant-based organic liquid fertilizer stocks and N-enriched water were prepared for this experiment at GreenF Aps, Denmark and at the Department of Engineering, Aarhus University, Denmark. Two fertilizer stocks were prepared by extraction of ensiled red clover (R_{ex}) and white mustard (M_{ex}) with acidic water (pH 4.2) (Fig. 1). Acidic water was produced by malting germinating seeds of broad bean (*Vicia faba*), triticale (× *Triticosecale*) and weeds (GreenF acid, GreenF Aps). Two fertilizer stocks were the liquid fractions from anaerobic digestion of a 1 : 3 mixture of either ensiled red clover and ensiled white mustard (RM_{di}) or the solid fraction from the acidic extraction of red clover and white mustard (RM_{ex+di}). Biomass was mixed with tap water at a ratio of 1 : 22 (based on dry weight) before digestion. RM_{ex+di} was therefore produced by a two-step treatment (acidic extraction followed by anaerobic digestion) to see if a better use efficiency of the original biomass was possible. The inoculum used for reactor start-up was collected from an anaerobic reactor located at Research Centre Foulum (Aarhus University, Denmark) treating cattle manure, grass and straw at thermophilic conditions (53°C). Two continuous stirred tank reactors were used in this work,

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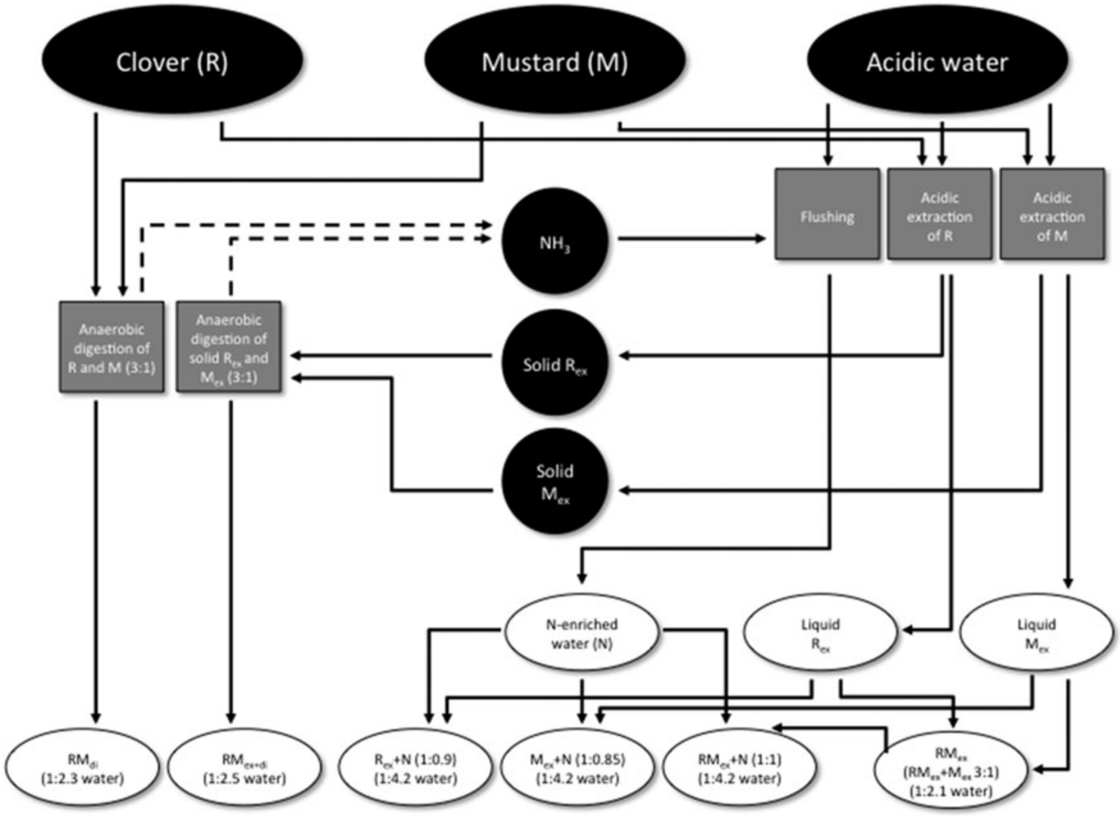

Fig. 1. Raw materials, fertilizer production methods and resulting liquid fertilizers. Acidic extraction of red clover (R_{ex}) and white mustard (M_{ex}), anaerobic digestion of red clover and white mustard (RM_{di}), anaerobic digestion of the solid fraction from R_{ex} and M_{ex} (RM_{ex+di}) and N-enriched water (N). Different colors indicate ■ raw material used, ■ method employed and ■ liquid fertilizer.

each with a 100 L working capacity. The two reactors used were similar in design and operated at a temperature of 50°C with an average hydraulic retention time of 30 days. The temperature was controlled by heating tubes. The reactors were operated for 65 days before the digestates were collected for this experiment. The fifth stock, N-enriched water was produced by flushing acidic water with ammonia produced by ammonia stripping of the solid fraction from anaerobically digested cattle manure at Research Centre Foulum (Aarhus University, Denmark). The ammonia could in a commercial setting come from stripping of the solid part after anaerobic digestion of mustard and clover as indicated in Figure 1. The stripping was done by drying the solid digestate in a grain drier and leading the steam to a 10-m^3 tank with acidic water where the ammonia in the steam was trapped. An overview of fertilizer production and fertilizer treatments is shown in Figure 1 and the obtained EC, pH and nutrient concentrations in the fertilizer stocks are shown in Table 1.

Experimental design and fertilizer treatments

For all treatments, 20 kg composted chicken manure (2–1–2 NPK, powdered, Farmergødning, Denmark) per cubic meter was mixed in the peat before filling pots. Nine treatments (six organic and three controls) with four replicates and 12 pots per replicate were established in a completely randomized design, where pots were randomly selected from within each replicate for later plant or growing medium analyses (described below). The control treatments were: (1) water (W), to determine the effect of only solid composted chicken manure mixed in before sowing and no liquid fertilizer was applied during growing period; (2) liquid inorganic fertilizer (IN) prepared from individual salts; and (3) chicken manure extract (3-1-2 NPK, granulated, Farmergødning, Denmark, soaked in water for 24 h 1 : 10 (w/w)] and filtered through a filter with mesh size 0.3 mm as organic control (C). Six organic treatments were prepared from the five plant-based organic liquid fertilizer stocks produced. Fertigation solutions RM_{di} and RM_{ex+di} were the original digestates diluted with water in the ratios 1 : 3 and 1 : 2.5 (v/v), respectively, before application. R_{ex} and M_{ex} (3 : 1 v/v) were mixed and diluted with water (1 : 2.1 v/v) to give RM_{ex}. All liquid fertilizers were filtered through a filter with mesh size 0.3 mm before being mixed with water. In order to increase the N concentration and dilute the high K and Cl concentrations, $\mathbf{R_{ex}}$, $\mathbf{M_{ex}}$ and $\mathbf{RM_{ex}}$ were individually mixed with N-enriched water to give R_{ex} + N (2 : 1.8 v/v), M_{ex} + N (2 : 1.7 v/v) and RM_{ex} + N (2 : 1.9 v/v) and then diluted with water (1 : 4.2 v/v) before application. A mixture of 75% tap water and 25% rainwater was used to prepare all fertigation solutions including W. Fertigation solutions were prepared to give a similar total-N in all fertigation treatments. An overview of nutrient concentrations in the given fertigation solutions is shown in Table 2.

EC, pH and nutrient content in fertilizers and in fertigation and peat solutions

The EC and pH of the growing medium solution were measured once a week from 0 days after fertigation start (DAF) to 21 DAF

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Table 1. Nutrient concentrations (mg L^{-1}), pH and EC (dSm^{-1}) of liquid fertilizer stocks produced with anaerobic digestion of red clover and white mustard (RM_{di}), anaerobic digestion of solid product after acidic extraction (RM_{ex+di}), acidic extraction red clover (R_{ex}) and white mustard (M_{ex}) and ammonia flushing in acidic water (N-enriched water).

Nutrients	EC	pH	NH_4-N	NO_3-N	N-min	P	K	Ca	Mg	S	Cl	Na	Fe	Zn	Mn	B	Cu	Mo
RM_{di}	13.0	8.0	688	3	691	55	2111	186	114	84	696	160	20.74	1.89	1.32	0.39	0.74	0.00
RM_{ex+di}	10.0	7.7	594	1	595	50	1358	233	115	65	801	199	13.26	2.85	1.12	0.24	0.34	0.00
R_{ex}	11.1	5.4	536	2	538	430	2549	439	219	122	347	38	18.30	2.50	3.70	0.83	0.10	0.005
M_{ex}	12.0	5.0	563	3	566	431	2505	642	402	223	631	80	19.00	3.50	3.87	0.64	0.05	0.005
N-enriched water	9.5	9.3	1400	2	1402	94	572	117	27	38	22	39	4.70	3.40	1.50	0.15	0.28	0.00

Table 2. Nutrient concentrations (mg L^{-1}), pH and EC (dS m^{-1}) of applied fertigation solutions from anaerobic digestates of red clover and white mustard (RM_{di}), anaerobic digestion of the solid product after extraction with acidic water (RM_{ex+di}), acidic extraction of red clover (R_{ex}) + white mustard (M_{ex}) (RM_{ex}), RM_{ex} + N-enriched water (RM_{ex} + N), R_{ex} + N-enriched water (R_{ex} + N), M_{ex} + N-enriched water (M_{ex} + N), chicken manure extract (C), inorganic fertilizer (IN) and water (W).

Treatments	pH	EC	NH_4-N	NO_3-N	N-min	P	K	Ca	Mg	S	Cl	Na	Fe	Zn	Mn	Cu	B	Mo
RM_{di}	8.0	3.3	231	4	235	19	423	116	33	31	155	49	4.0	0.4	0.4	0.1	0.1	0.01
RM_{ex+di}	7.9	3.1	217	4	221	19	323	128	35	30	147	55	3.6	0.5	0.4	0.1	0.3	0.01
RM_{ex}	6.1	4.7	215	4	219	131	762	227	72	44	162	31	6.6	1.1	1.6	0.05	0.4	0.01
RM_{ex} + N	6.6	2.9	202	4	206	47	330	141	28	32	77	23	2.5	0.6	0.5	0.05	0.2	0.01
R_{ex} + N	6.8	2.9	200	4	204	43	340	132	29	29	77	24	1.8	0.5	1.5	0.03	0.2	0.01
M_{ex} + N	6.1	2.8	177	4	181	45	315	159	27	37	102	27	2.4	0.9	1.0	0.03	0.2	0.01
C	7.1	2.4	109	4	113	28	439	63	11	88	222	78	3.9	1.4	0.2	0.3	0.3	0.1
IN	5.9	2.0	20	185	205	34	253	156	36	78	40	21	1.5	0.4	1.3	0.2	0.3	0.01
W	7.8	0.6	0.2	4	4	2	7	116	9	27	37	19	0.1	0.1	0.1	<0.01	0.1	<0.01

for each replicate by a pH and EC meter (Model no. HI 9813, HANNA Instruments Inc., Romania). Samples of fertilizers, fertigation solutions immediately following the preparation and growing medium solutions were taken on 21 DAF and analyzed for NH_4, NO_3, P, K, Ca, Mg, S, Cl, Na, Fe, Zn, Mn, Cu, B and Mo (Eurofins, Denmark). Growing medium solution samples were collected from one to two randomly selected pots (10–20 ml) for pH and EC measurements and from six to nine pots (150 ml) for nutrient analysis by suction of individual pots with a vacuum 1h after running a fertigation treatment.

Biomass yield and nutrient content in plant materials

Growth parameters were determined on plants from two pots per replicate at 24 DAF. Eight young fully developed leaves from each pot (one per plant) were harvested and leaf area, fresh weight and dry weight were determined to calculate the specific leaf area (SLA, $cm^2\ g^{-1}$ DM). The remaining aboveground biomass was harvested and the number of leaves, fresh weight and the total leaf area were recorded (LI-3100, Li-Cor Inc., Lincoln, NE, USA). The fresh biomass was oven-dried at 70°C for 48 h to determine dry weight. Plant material from another four pots per replicate was harvested, dried and mixed with the dry matter from the two pots above, and analyzed for total N, P, K, Ca, Mg, S, Cl, Na, Fe, Zn, Mn, Cu and B (Agrolab, Germany).

Statistical analysis

The statistical data analysis was done using the GLM procedure of SAS (Statistical Analysis System, version 9.2, SAS Institute, Cary, CN, USA). Mean values were used in the statistical analysis if measurements on several pots were taken within a replicate. Biomass yield, leaf area, water content, SLA and nutrient concentration in the growing medium and plant material were analyzed using one-way ANOVA. For pH and EC two-way interaction between treatment and day of measurement was identified by two-way ANOVA and multiple comparisons of means were done by using proc PLM (partial linear model). Tukey's studentized range test was applied to determine significant differences between treatments at the $P < 0.05$ level of significance.

Results

Nutrient concentrations in fertilizers

Both the source materials (red clover and white mustard) and production methods used (acidic extraction and anaerobic digestion) influenced the concentrations of nutrients in the resulting plant-based liquid fertilizer stocks (Table 1). Apart from N-enriched water, plant-based fertilizer stocks had high K concentrations compared with N irrespective of source material and production method used. Fertilizer stocks prepared by acidic extraction had higher concentrations of K, P, Ca, Mg, S, Mn and B and lower concentrations of N, Cl, Na and Cu than with RM_{di} and RM_{ex+di} fertilizer stocks, and the pH in RM_{di} and RM_{ex+di} fertilizer stocks was 2–3 units higher than after acidic extraction (RM_{ex}). Higher concentrations of Ca, Mg, S, Cl, Na, Zn and Mn were found in fertilizer stocks prepared from white mustard compared with red clover, whereas the concentration of other nutrients was similar in all fertilizer stocks.

Plant growth parameters

Parsley shoot dry weight ($P < 0.001$) and total leaf area ($P < 0.001$) were higher in IN than RM_{ex}, RM_{di} and RM_{ex+di} (Table 3). However, when R_{ex}, M_{ex} or RM_{ex} were combined with N-enriched water ($R_{ex} + N$, $M_{ex} + N$ or $RM_{ex} + N$), dry weight and leaf area were not different than IN, though values were numerically intermediate between IN and organic treatments without N-enriched water. The number of leaves and the specific leaf area did not differ significantly between fertigation treatments (Table 3). Neither did the production method for red clover and white mustard fertilizer (RM_{ex} versus RM_{di} and RM_{ex+di}) significantly influence plant growth parameters. The shoot dry weight, leaf area, leaf number and specific leaf area were found to be lower in W than in all fertigation treatments ($P < 0.001$).

pH and EC in growing medium solutions

The pH and EC of growing medium solutions were affected by the type of fertilizer and date of measurement (Fig. 2; $P < 0.001$). The pH increased gradually throughout the experimental period in all treatments (Fig. 2a and b) but the dynamics differed. For example, in all organic treatments and W, pH increased by one unit

Table 3. Leaf number, leaf area, dry weight, leaf water content and specific leaf area of parsley grown with liquid fertilizers from anaerobic digestion of red clover and white mustard (RM_{di}), anaerobic digestion of the solid product after extraction with acidic water (RM_{ex+di}), acidic extraction of red clover ($\mathbf{R_{ex}}$) + white mustard ($\mathbf{M_{ex}}$) (RM_{ex}), $\mathbf{RM_{ex}}$ + N-enriched water (RM_{ex} + N), $\mathbf{R_{ex}}$ + N-enriched water (R_{ex} + N), $\mathbf{M_{ex}}$ + N-enriched water (M_{ex} + N), chicken manure extract (C), inorganic fertilizer (IN) and water (W). Means ($n = 4$) within each row followed by different letters are statistically different between fertigation strategies ($P < 0.05$).

Treatments	Leaf number	Leaf area (cm^{-2} pot^{-1})	Dry weight (g pot^{-1})	Water content (g g^{-1} DM)	SLA (cm^2 g^{-1} DM)
RM_{di}	109^a	4248^b	6.9^b	7.4^b	455^{ab}
RM_{ex+di}	107^a	4135^b	6.4^b	8.1^{ab}	480^a
RM_{ex}	108^a	4089^b	6.4^b	7.4^b	490^a
RM_{ex} + N	110^a	4720^{ab}	7.4^{ab}	8.5^{ab}	478^a
R_{ex} + N	112^a	4804^{ab}	7.6^{ab}	8.6^{ab}	472^{ab}
M_{ex} + N	110^a	4465^{ab}	7.3^{ab}	8.6^{ab}	452^{ab}
C	114^a	4904^{ab}	7.5^{ab}	9.1^a	460^{ab}
IN	115^a	5237^a	8.7^a	7.7^b	433^{ab}
W	86^b	1243^c	2.5^c	4.3^b	383^b

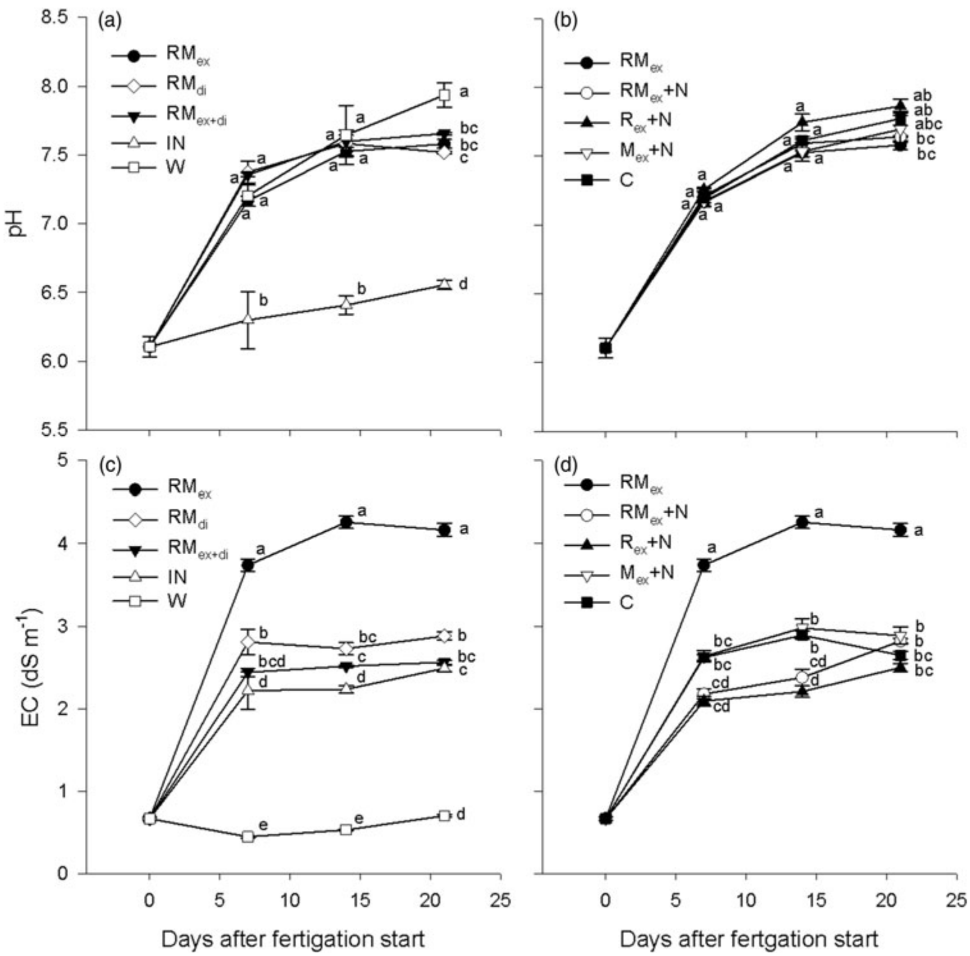

Fig. 2. The pH (a, b) and EC (c, d) in growing medium solutions after the application of liquid organic fertilizers prepared by different extraction methods of different raw materials. Acidic extraction of red clover (R_{ex}) and white mustard (M_{ex}) combined to give (RM_{ex}), anaerobic digestion of red clover and white mustard (RM_{di}) or of the solid product after acidic extraction (RM_{ex+di}), RM_{ex} + N (N = N-enriched water), R_{ex} + N, M_{ex} + N, chicken manure extract (C), inorganic fertilizer (IN) and water (W). Vertical bars indicate standard error of the mean ($n = 3$) and different letters indicate statistical difference between fertigation strategies within individual dates ($P < 0.05$).

between 0 and 7 DAF, further increased by 0.2–0.5 units at 14 DAF and subsequently stabilized, except in W where it continued to increase until the end of the experiment. In IN, the pH increased continuously throughout the experiment. Although the pH of the acidic extracted fertilizer RM_{ex} was lower than in RM_{di} and RM_{ex+di}, this was not reflected in the pH of the growing medium solution (Fig. 2a).

Fertigation with RM_{ex} resulted in a higher EC than with the other treatments (Fig. 2c and d), and the addition of N-enriched water (RM_{ex} + N) significantly reduced EC. During the first 14 days the EC in the growing medium solutions of R_{ex} + N and RM_{ex} + N was similar to IN and lower than in the other organic treatments. EC in RM_{di}, RM_{ex+di} and N-enriched treatments was similar to C and generally a little higher than IN. The EC in W remained between 0.45 and 0.71 dS m^{-1} throughout the experimental period.

Nutrient concentrations in growing medium solutions

The liquid fertilizers had a large influence on the nutrient composition of the growing medium (Table 4). The standard inorganic fertilizer prepared according to crop need from individual salts resulted in high N-min, NO_3-N, Ca, S, Mn and B concentrations and low K and Cl concentrations compared with the organic fertilizers. The organic control treatment based on an extract from composted chicken manure (C) was very high in Cl, Na, Fe, Zn and Cu and low in Ca and Mg compared with both the other organic treatments and IN. Of the plant-based organic fertilizers, RM_{di} and RM_{ex+di} were relatively high in NO_3-N, S and Na and low in N-min and NH_4-N. RM_{di} also had a high Fe concentration similar to C. RM_{ex} resulted in very high concentrations of P, K and Mg compared with IN, C, RM_{di} and RM_{ex+di}.

Nutrient concentrations in leaves

At the end of the experiment, the leaf concentrations of total-N, P, K, Fe and Zn were similar in all treatments receiving fertigation and generally higher than in W (Table 5). Despite differences in nutrient concentrations in the growing medium, the leaf concentrations did not vary much with the type of applied organic fertilizers. Fertigation with RM_{di} and RM_{ex+di} resulted in high leaf concentrations of only B and Cu compared to RM_{ex}, but high concentrations of B and Cu were also found in leaves from C. The leaf Mn concentration was generally higher in the

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Table 4. Concentrations (mg L^{-1}) of nutrients in the growing medium solution 21 days after application of liquid fertilizers from anaerobic digestion of red clover and white mustard (RM_{di}), anaerobic digestion of solid product after extraction with acidic water (RM_{ex+di}), acidic extraction of red clover (R_{ex}) + white mustard (M_{ex}) (RM_{ex}), RM_{ex} + N-enriched water (RM_{ex} + N), R_{ex} + N-enriched water (R_{ex} + N), M_{ex} + N-enriched water (M_{ex} + N), chicken manure extract (C), inorganic fertilizer (IN) and water (W). Means ($n = 4$) within each row followed by different letters are statistically different between fertigation strategies ($P < 0.05$).

Treatments	NH_4-N	NO_3-N	N-min	P	K	Ca	Mg	S	Cl	Na	Fe	Zn	Mn	Cu	B
RM_{di}	110^{de}	31^{b}	141^{c}	30^{b}	508^{b}	128^{abc}	38^{abc}	36^{bc}	183^{b}	61^{b}	3.0^{a}	0.64^{b}	0.49^{b}	0.03^{b}	$<0.1^{a}$
RM_{ex+di}	87^{e}	47^{b}	134^{c}	26^{b}	403^{bc}	118^{abc}	37^{bc}	35^{bc}	168^{bc}	69^{b}	2.3^{b}	0.51^{bc}	0.35^{bc}	0.03^{b}	$<0.1^{a}$
RM_{ex}	197^{a}	6^{c}	203^{b}	60^{a}	805^{a}	123^{abc}	58^{a}	16^{c}	163^{bc}	37^{c}	1.3^{c}	0.17^{cd}	0.27^{bcd}	$<0.1^{b}$	$<0.1^{a}$
RM_{ex} + N	169^{ab}	6^{c}	175^{bc}	38^{b}	488^{b}	145^{ab}	39^{abc}	29^{c}	116^{cd}	38^{c}	0.82^{cd}	0.11^{cd}	0.39^{bc}	0.03^{b}	$<0.1^{a}$
R_{ex} + N	147^{bc}	6^{c}	153^{bc}	27^{b}	395^{bc}	100^{bc}	$25b^{cd}$	12^{c}	94^{de}	29^{cd}	0.45^{de}	$<0.1^{d}$	0.14^{cd}	$<0.1^{b}$	$<0.1^{a}$
M_{ex} + N	182^{a}	6^{c}	188^{b}	29^{b}	368^{bc}	118^{abc}	28^{bcd}	8^{c}	128^{cd}	35^{cd}	0.3^{de8}	$<0.1^{d}$	0.22^{bcd}	$<0.1^{b}$	$<0.1^{a}$
C	125^{cd}	6^{c}	131^{c}	44^{ab}	520^{b}	75^{c}	18^{cd}	71^{ab}	240^{a}	92^{a}	3.3^{a}	1.05^{a}	0.35^{bc}	0.18^{a}	$<0.1^{a}$
IN	29^{f}	226^{a}	255^{a}	28^{b}	273^{c}	178^{a}	40^{ab}	73^{a}	70^{e}	32^{cd}	0.76^{cd}	0.44^{bc}	0.83^{a}	0.07^{b}	0.14^{a}
W	2^{f}	6^{c}	8^{d}	3^{c}	27^{d}	110^{abc}	13^{d}	33^{c}	59^{e}	25^{d}	$<0.1^{e}$	$<0.1^{d}$	0.033^{d}	$<0.1^{b}$	$<0.1^{a}$

Table 5. Concentrations (mg L^{-1}) of nutrients in parsley leaves after application of liquid fertilizers from anaerobic digestion of red clover and white mustard (RM_{di}), anaerobic digestion of the solid product after extraction with acidic water (RM_{ex+di}), acidic extraction of red clover (R_{ex}) + white mustard (M_{ex}) (RM_{ex}), RM_{ex} + N-enriched water (RM_{ex} + N), R_{ex} + N-enriched water (R_{ex} + N), M_{ex} + N-enriched water (M_{ex} + N), chicken manure extract (C), inorganic fertilizer (IN) and water (W). Means ($n = 4$) within each row followed by different letters are statistically different between fertigation strategies ($P < 0.05$).

	Macronutrients (g kg^{-1} DM)								Micronutrients (mg kg^{-1} DM)				
Treatments	Total-N	P	K	Ca	Mg	S	Na	Cl	Fe	Zn	Mn	Cu	B
RM_{di}	42^{a}	5.9^{ab}	72^{a}	9.4^{ab}	3.29^{abc}	3.14^{a}	3.16^{bcd}	13.6^{b}	56^{a}	69^{a}	59^{a}	3.6^{a}	18.2^{bc}
RM_{ex+di}	45^{a}	5.6^{ab}	69^{a}	9.6^{ab}	3.31^{abc}	2.96^{a}	3.63^{abc}	14.0^{b}	51^{ab}	67^{a}	57^{a}	3.3^{ab}	19.7^{b}
RM_{ex}	41^{a}	6.2^{a}	78^{a}	7.2^{b}	2.85^{bc}	2.86^{a}	2.55^{cd}	16.1^{b}	49^{ab}	54^{a}	54^{ab}	1.6^{c}	13.7^{d}
RM_{ex} + N	45^{a}	7.2^{a}	75^{a}	9.6^{ab}	3.39^{abc}	3.05^{a}	2.81^{cd}	16.3^{b}	56^{a}	54^{a}	42^{abc}	1.3^{c}	14.2^{d}
R_{ex} + N	44^{a}	7.1^{a}	76^{a}	10.2^{ab}	3.55^{ab}	3.35^{a}	2.83^{cd}	14.7^{b}	59^{a}	55^{a}	45^{abc}	1.3^{c}	16.3^{cd}
M_{ex} + N	43^{a}	6.8^{a}	73^{a}	9.9^{ab}	3.43^{abc}	2.88^{a}	2.77^{cd}	14.3^{b}	52^{ab}	50^{a}	43^{abc}	1.3^{c}	14.0^{d}
C	41^{a}	5.6^{ab}	68^{a}	7.2^{b}	2.57^{c}	3.49^{a}	4.09^{ab}	28.6^{a}	48^{ab}	66^{a}	38^{bc}	3.5^{a}	26.3^{a}
IN	40^{a}	6.6^{a}	72^{a}	12.4^{a}	3.94^{a}	2.90^{a}	2.33^{d}	6.6^{c}	60^{a}	52^{a}	30^{c}	1.9^{bc}	27.0^{a}
W	15^{b}	4.1^{b}	36^{b}	10.5^{a}	2.64^{c}	2.62^{a}	4.44^{a}	14.5^{b}	35^{b}	19^{b}	42^{abc}	1.3^{c}	20.7^{b}

treatments receiving plant-based organic fertilizers than with IN. The leaf Ca and Mg concentrations were about 1.5 times higher in IN than in C and RM_{ex}, but their concentrations did not differ significantly in the organic treatments. The Cl concentration in C was 4.5 times higher than in IN and twice as high as in the other organic treatments and W. The Na concentration was furthermore high in C and W (Table 5).

Discussion

The effect of extraction method on stock fertilizer nutrient content

In this study, organic liquid fertilizer stocks were produced from ensiled red clover and white mustard by either acidic extraction, anaerobic digestion or both. Many plant- and animal-based sources have previously been used to produce organic liquid fertilizers using extraction, fermentation, anaerobic digestion or other methods (Bujoczek et al., 2000; Gross et al., 2008; Martínez-Alcántara et al., 2016) and variations in composition and concentration of nutrients in the resulting liquid fertilizers have been observed (Gross et al., 2008; Hartz et al., 2010). Generally, N-min (sum of ammonium and nitrate) in plant-based liquid fertilizers is low compared with the concentrations of available K and Cl (Martínez-Alcántara et al., 2016; Pokhrel et al., 2017a), which reflects their biochemical function in plant tissue. Most of the N in plant tissue is bound in organic form, mainly as amino acids and proteins, and needs microbial breakdown (mineralization) to become available in ionic form. In contrast, most of the K and Cl is held in ionic forms in plant cells and is therefore easily extractable (Römheld and Kirkby, 2010; Marschner, 2011). The higher N-min to K ratio in RM_{di} than RM_{ex} fertilizer stocks could be due to a more extensive microbial degradation of N during anaerobic digestion than with acidic extraction (Table 1). In contrast to N; P and Ca were higher in fertilizer stocks derived from acidic extraction than anaerobic digestion could be due to easier to the breakdown of carbon bound P and Ca than N by acidic water. Only the K and Fe concentrations were lower in RM_{ex+di} than in RM_{di} even though nutrients had already been extracted from the biomass once during the preparation of RM_{ex}. This can be explained by the two-step treatment (acidic extraction followed by anaerobic digestion) facilitating the breakdown of more complex compounds in the plant tissue than by either method alone.

The N:K ratio in fertigation solutions was higher than in the fertilizer stock before dilution, which could be due to mineralization of some organic N during the preparation of the solutions and during fertilizer stock storage (Tables 1 and 2). Martínez-Alcántara et al. (2016) reported that more than 80% of the N content in newly extracted liquid fertilizers is in the organic form, which can be easily mineralized. Fertigation solutions prepared from fertilizer derived from acidic extraction (RM_{ex}) contained higher concentrations of most macro- and micronutrients (except N, Na and Cu) and higher EC than fertigations solutions prepared from RM_{di} and RM_{ex+di} stocks. This was due to a low concentration of N relative to other nutrients in RM_{ex} because fertigation solutions were prepared to supply similar concentrations of total N. Although the NO_3 concentration in the growing medium solutions of RM_{di} and RM_{ex+di} was higher than in the respective fertigation solutions, the N-min was lower compared with RM_{ex} (Tables 2 and 4). This could be attributed to the high pH (ca. 8) of both RM_{di} and RM_{ex+di} fertilizer stocks and fertigation solutions, which may have increased both the ammonia volatilization rate and nitrification after the preparation of fertigation solutions (Robinson et al., 2014). The NO_3 concentration in the fertigation solutions was slightly higher than in fertilizer stocks, which was probably due to nitrification during storage of fertilizer stocks because the stocks were only analyzed at the start of the experiment.

The effect of extraction method on plant growth

Compared with IN and C the application of RM_{ex}, RM_{di} and RM_{ex+di} reduced dry weight and total leaf area (Table 3). The lower biomass yield from RM_{ex} was likely due to an elevated EC in the growing medium solution, caused by an excess concentration of K. High K concentrations and EC previously been reported when using organic liquid fertilizer to meet crop total-N requirements (Pokhrel et al., 2017a). In this study, the root zone EC for plants receiving RM_{ex} was approximately twice as high as the upper threshold for optimal parsley growth reported by Álvaro et al. (2015). It is well known that a high EC has negative effects on water and nutrient uptake due to lower water potential, resulting in reduced crop growth (Ouni et al., 2014; Rameshwaran et al., 2016). Moreover, excessive K and NH_4 concentrations influence the cation balance and result in reduced Ca and Mg uptake, which was supported from the numerically lower concentration of Ca and Mg in the plant materials in organic treatments (Li et al., 2013). The lower N-min in the growing medium, potentially resulting from high pH-related N losses after fertigation preparation, could also partly explain the reduced yield of RM_{di} and RM_{ex+di} compared with IN and N-enriched treatments. High pH has previously been found to lead to ammonia volatilization, at the expense of plant uptake (Kai et al., 2008). High EC and imbalance nutrient compositions in the root zone, and low root growth (data not shown) may also be responsible for lower yield from plant-based fertilizers without combined N-enriched water compared to with N-enrich water combined treatments.

The effect of N-enriched water on plant growth

The fresh weight, dry weight and leaf area increased by up to 35% when R_{ex}, M_{ex} and RM_{ex} were combined with N-enriched water compared with RM_{ex} alone, which was likely due to an improved nutrient composition and reduced EC in the growing medium. N-min levels in fertigation solutions and growing medium solutions were similar for RM_{ex} and N-enriched treatments, but the concentration of K was reduced by ca. 50% in N-enriched treatments, which not only reduced EC but also increased Ca/K and Mg/K ratios.

Despite significantly higher pH and NH_4 concentrations in the growing medium in C and N-enriched water treatments compared with IN, parsley plants had similar fresh biomass, dry weights and leaf areas. This indicates that the lower yield in RM_{ex}, RM_{di} and RM_{ex+di} may not be due only to negative effects of a high pH and high NH_4 concentration in the growing medium. This argument is supported by the lower yield in RM_{di} and RM_{ex+di} despite a higher NO_3 concentration in the growing medium solution compared with C and the N-enriched treatments. It could be that when high pH and NH_4 concentrations occur simultaneously, the negative individual impact of these factors is reduced. Previous studies have demonstrated that acidification of the rhizosphere is one of the fundamental causes of NH_4 toxicity to plant growth, and may be counteracted by applying fertigation solutions with a high pH (Dijk and Eck, 1995; Britto and Kronzucker, 2002). Therefore, high pH in fertigation solutions concomitant with high NH_4 concentrations may be beneficial. Achieving similar yields with C and IN is inconsistent with a previous study where a lower yield was found in C than IN (Pokhrel et al., 2017a), but could be explained by the slightly higher concentrations of most of the macro- and micronutrients in the applied and growing medium solutions in the present experiment.

Conclusion

Acidic extraction of red clover and white mustard silage resulted in higher concentrations of most of the macro- and micronutrients, except N, than anaerobic digestion of the same biomasses leading to high EC in the RM_{ex} fertigation and growing medium solutions. Fertigation solutions prepared from anaerobic digestates had a high pH of ca. 8. Both types of fertilizer resulted in low biomass yield of parsley compared with chicken manure extract and inorganic fertigation. Our results demonstrate that the yield of organic greenhouse parsley achieved with a chicken manure extract or a combination of plant-based fertilizers with N-enriched water was similar to that in a conventional system. In order to meet an increasing demand for organic fruits and vegetables grown in a greenhouse, liquid fertilizers are necessary, but only a few commercially available liquid fertilizers approved for organic production in Denmark. Finding of this study could be increased selection options of liquid fertilizers for organic growers because some fertilizers producers have been already started to produce acidic extracted plant-based fertilizers in Denmark. N-enriched has a potential to being approved as organic if produced from organic sources. The combinations of plant-based fertilizers and N-enriched water resulted in excellent plant growth of parsley, which widening the choice of certified liquid organic fertilizers in the market.

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