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Investigating the Effects of Fish Effluents as Organic Fertilisers on Basil (*Ocimum basilicum*)

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Abstract: Whilst the potential of fish effluents as nutrient sources for crop production has been demonstrated, their use in the European Union remains prohibited in organic farming. In this study, we investigate the efficacy in greenhouse basil cultivation of two types of fish effluents (filtered 'fish water' and unfiltered 'fish sludge') from an aquaponic system, and assess their role in maintaining and enhancing soil fertility as well as their potential to create a 'living soil', which are two of the prerequisites for organic certification in the EU. To evaluate the contribution of fish effluents to plant growth in comparison with soil nutrients, basil plants were grown in pots containing two types of substrate: compost-free (without organic matter) and with compost (with organic matter). The results indicate that fish water and fish sludge demonstrate significant potential as fertilisers and outperform compost in certain parameters, such as plant biomass. The results also align with existing literature by demonstrating the positive impact of compost on soil microbial diversity, underscoring its role in fostering plant health. Although the treatments did not show differences in microbial composition at the genus level, the higher microbial diversity observed following fish effluent application highlights its potential for promoting 'living soil'. This research underscores the need for continued exploration of the implications of compost application in conjunction with fish effluent fertilisation on soil microbial communities and the production of specialty crops such as herbs.

Keywords: basil; fish; effluents; aquaculture; aquaponics; organic; sludge

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1. Introduction

Whilst the potential of wastes from aquaculture farms, termed fish effluents, as a nutrient source for crop production has been demonstrated, their use is not sanctioned in organic farming within the prevailing European regulatory framework—Regulation (EU) 2018/848 [1], which lays down the rules for organic production, and Regulation (EU) 2023/121 [2], which specifies the types of fertilisers that are authorised. However, using fish wastewater as a fertiliser can benefit the environment in several ways. By diverting nutrient-rich effluent from aquatic ecosystems, it can help mitigate eutrophication caused by excess nitrogen and phosphorus [3]. Moreover, repurposing fish sludge in agriculture reduces the carbon footprint associated with manufacturing and transporting synthetic fertilisers [4]. Economically, fish wastewater can be cost-effective for growers located near aquaculture facilities, as it lowers reliance on commercial fertilisers and cuts waste disposal costs [5]. Fish manure (sludge) has a chemical composition akin to

livestock manure [6], and fish effluents (sludge or fish tank water) have been used successfully to fertilise various food crops, including tomato [7–13], pepper [14], chicory [15], lettuce [16–19], cucumber [20], potato, soybean, and onion [21]. Up to 50% (in dry matter) of the feed consumed by fish is expelled as solids, forming sludge [22], and a significant portion of the nutrients introduced into an aquaculture system is wasted through the routine discharge of this nutrient-rich matter, which is primarily composed of fish excrement and unconsumed feed [23]. However, in most aquaculture farms, the sludge is released as sewage, and only occasionally is it dried and repurposed as fertiliser [24]. Aquaculture sludge holds considerable promise for enhancing plant growth due to its nutrient content [25], and a substantial amount of the essential macro- and micronutrients can be derived from this solid waste, which has been found to contain 7–32% of the total nitrogen and 30–84% of the total phosphorus present in wastewater [26]. The sludge is particularly abundant in phosphorus, a crucial macronutrient for plant growth which has been recognised as a Critical Raw Material in the EU due to its high supply risk, and conventional filtration in aquaponic systems eliminates over 80% of it [27]. The nutrient composition of filtered aquaculture effluents differs from that of sludge, with the latter offering a much richer and broader nutritional spectrum. Indeed, in comparison to sludge, filtered aquaculture effluent typically lacks phosphorus, potassium, calcium, and micronutrients, notably iron, molybdenum, and manganese [10].

Should the fish feed be organic in provenance, then the application of fish effluents could supply crops with organic fertiliser through a natural process observed in aquatic environments, where fish effluents contribute to plant growth in ponds and lakes [28]. Additionally, recycling wastewater that would otherwise pollute water bodies could offer a potential remedy for the environmental challenges associated with the eutrophication of aquatic ecosystems [29]. In this manner, the use of fish effluents could also fulfil an environmental conservation role by partially replacing the need for synthetic fertilisers, the production of which consumes significant energy [30], often sourced in environmentally damaging ways, such as phosphorus mining [31,32].

In this study, the efficacy of two types of fish effluents (filtered and unfiltered) from an aquaponic system was tested to grow basil in greenhouse conditions. The main objectives were to test their viability as a source of fertiliser for the greenhouse cultivation of herbs, and to investigate their potential for maintaining and enhancing soil fertility and for enhancing the production of 'living soil', which are conditions that need to be fulfilled for organic certification in Europe. In order to ascertain the role of fish effluents in the growth of the plants as opposed to the nutrients already present in soil, the basil plants were grown in pots filled with two types of substrate, one compost-free and the other with compost. The filtered fish water is hereafter referred to as 'fish water', the unfiltered fish water as 'fish sludge' and the water from the tap as 'tap water'. When referring to both fish water and fish sludge, they are referred to as 'fish effluents'. A key novelty of this study is its direct comparison of compost and fish effluents as fertilisers for the cultivation of basil in greenhouse conditions. By evaluating plant growth and soil microbial properties across treatments, this work provides a unique perspective on the relative advantages of compost versus fish effluents, advancing our understanding of how these fertiliser strategies can be optimised for both high-value crop production and soil health in sustainable farming systems.

2. Materials and Methods

2.1. Outline

The experiments took place inside a 7.2 × 3.4 m (24 m^2) greenhouse located on the second floor of the School of Design of the University of Greenwich in London, UK. The experiment started on July 13th and ended on 26 August 2021, lasting a total of 44 days.

Basil plants were grown in plastic pots (one plant per pot) placed within plastic trays under grow lights. Two types of potting substrates, one with compost and one without, and two fish effluent types (fish water and fish sludge) were used in combination.

Tap water was sourced from the University's potable water system, whilst fish effluents were obtained from the University of Greenwich aquaponics greenhouse. The greenhouse accommodated a floating raft aquaponic system stocked with approximately 300 Nile tilapia (*Oreochromis niloticus*), alongside a variety of plant species such as beans, squash, mint, tomato, cucumber, okra, fern, turmeric, cape gooseberry, melon, and various ornamentals. The Nile tilapia, weighing between 100 and 500 g, were fed twice daily, once in the morning and once in the afternoon, with Aller Aqua Primo 6 mm sinking pellet feed (37% Crude Protein, 12% Crude Fat, 32.5% Nitrogen-free Extracts, 7% Ash, 3.5% Fibre, 1% Phosphorus, 19.6 MJ Gross Energy, 16 MJ Digestible Energy), totalling 500 g of feed per day.

Most of the fish sludge was removed from the water before it entered the plant (hydroponic) compartments. The first step of filtration involved the clarifier, where the water from the fish tanks was drawn from the bottom, and as it flowed into the clarifier, the settleable solids dropped. To ensure that almost all large solids were removed before the water entered the hydroponic section, single or multiple tanks with orchard netting were employed, achieving almost total removal of solids. The fish sludge was collected from the bottom of the clarifiers, while the fish water was taken from the sump, a compartment where the filtered water flowed and was further aerated before heading to the plant units.

2.2. Crop Choice

F1 hybrid basil *Ocimum basilicum* 'Aroma 2' was used in the experiment, grown from seed. Basil is fast-growing and of high value, and as a result has become one of the main crops grown in aquaponics in both commercial and research settings [33–38]. Basil is also a herb, and herbs constitute an exception to rule 1.1 of Annex II of Regulation (EU) 2018/848 on the mandatory connection with the subsoil and bedrock—this means that basil can be grown in pots and still be certified as organic in the EU. Furthermore, the morphology of the crop allows for easy measurement of growth parameters such as height and stem diameter, the leaves are easily collectable for further analysis, and the crop has been widely studied and analysed for biomass and nutrient content when subjected to a variety of different growing conditions. Finally, the 'Aroma 2' cultivar is one of the most popular for hydroponic growers of basil, chosen for its uniformity, fast growth, classic flavour and aroma profile, as well as resistance to *Fusarium* [39].

2.3. Treatments

Two substrates and three fertilisation regimes were combined for a total of six treatments, each replicated four times: compost-free substrate irrigated with tap water (treatment IT); compost-free substrate irrigated with fish water (treatment IF); compost-free substrate irrigated with fish water and every third day with fish sludge (treatment IFS); compost substrate irrigated with tap water (treatment CT); compost substrate irrigated with fish water (treatment CF); and compost substrate irrigated with fish water and every third day with fish sludge (treatment CFS). The pots were randomly distributed in four blocks. The composition of the tap water was established, and the composition of the two fish effluents was analysed (Table 1). All plants were irrigated from the top and given 10 mL of either tap water or fish effluent per day.

Table 1. Parameters of the tap water, fish water, and fish sludge.

Parameter	Tap Water [1]	Fish Water [2]	Fish Sludge [2]
pH	7.60 [3]	6.7	5.8
Calcium carbonate concentration	323 ppm	-	-
Conductivity at 20 °C	594 µS/cm	-	-
Conductivity at 25 °C	-	791 µS/cm	1020 µS/cm
Turbidity	<0.09 FTU	-	-
Ammonium as NH_4	0.12 mg/L	-	-
Nitrate as NO_3	27.2 mg/L	44.3 mg/L	69.6 mg/L
Nitrate/nitrite calculation	0.55 mg/L	-	-
Nitrite as NO_2	0.013 mg/L	-	-
Hardness (total) as $CaCO_3$	244 mg/L	-	-
Iron as Fe	3.6 µg/l	-	-
Magnesium	4.3 mg/L	12.33 mg/L	18.34 mg/L
Alkalinity (HCO_3)	-	36 mg/L	13 mg/L
Sulphate (SO_4)	-	161.8 mg/L	204.4 mg/L
Boron	-	0.01 mg/L	0.03 mg/L
Sodium	-	44.0 mg/L	50.5 mg/L
Chloride	-	59.2 mg/L	69.8 mg/L
Phosphorus as P	-	4.4 mg/L	12.8 mg/L
Potassium	-	1.5 mg/L	3.5 mg/L
Calcium	-	112.6 mg/L	136.4 mg/L
Carbonate	-	<10 mg/L	<10 mg/L
Total dissolved solids	-	553.7 mg/L	714 mg/L

[1] Water parameters for postcode SE10 9BD obtained from https://www.thameswater.co.uk/help/water-quality/check-your-water-quality#/results/SE109BD (accessed on 2 June 2021). [2] Analysed by NRM Labs on 11 June 2021. NRM Labs, Coopers Bridge, Braziers Lane, Winkfield Row, Bracknell RG42 6NS, United Kingdom. [3] pH measured consistently at the University of Greenwich shows a value of approximately 8.40.

2.4. Substrate Mixes

A review of the physical, chemical, and biological properties of soil ecosystems revealed that there appears to be no clear definition of what constitutes soil; this is reflected in Regulation (EU) 2018/848 [1], where no definition of 'soil', nor of what constitutes soil, is given. With regard to the experimental setup, it was considered that a comparison between a substrate mix with compost and one without compost would be useful in order to determine the influence of compost on the substrate that was exposed to fish effluents. It was therefore decided to include both substrate types, one nutrient-rich where compost was included, and one nutrient-poor without compost. To ensure that the substrates were as similar as possible in composition and texture, both substrates were manufactured from raw ingredients using the Royal Horticultural Society guidelines for creating a John Innes Potting Compost [40]. Two substrate types were thus devised, using only materials approved in organic certification: a substrate without compost, having 3 parts loam, 3 parts sand, and 5 parts coir; and a substrate with compost, having 3 parts loam, 3 parts sand, 3.5 parts coir, and 1.5 parts compost (13.6%). The two substrate types were analysed for nutrients and physicochemical parameters (Table 2).

All nutrient values, except for manganese and calcium, were higher in the compost substrate, reflecting the higher nutrient load supplied by the compost.

Table 2. Values for pH, elements, and physical parameters of the two initial substrate types used.

Parameter	Compost-Free Substrate	Compost Substrate
pH	8	7.81
P	18 mg/L	36.80 mg/L
K	218.35 mg/L	538.20 mg/L
Mg	55.90 mg/L	111.65 mg/L
Mn	7.88 mg/L	7.32 mg/L
Cu	1.56 mg/L	1.97 mg/L
B	0.97 mg/L	1.35 mg/L
Na	37.50 mg/L	85 mg/L
Zn	2.03 mg/L	5.40 mg/L
Ca	1835 mg/L	1700 mg/L
Av Fe	11.96 mg/L	23.70 mg/L
Nitrate-N	61.44 kg/ha	361 kg/ha
Ammonium-N	2.80 kg/ha	7.96 kg/ha
Soil mineral nitrogen	64 kg/ha	369 kg/ha
Organic matter LOI [1]	5.44%	6.22%
Av SO_4	47.02 mg/L	56.86 mg/L
Cation exchange capacity	13.60 meq/100 g	14.7 meq/100 g

[1] Loss on ignition.

2.5. Experimental Setup and Growth Conditions

A total of five 8 cm pots were placed in each tray (Figure 1), and the trays (dimensions: 21 × 16 × 5 cm) for each treatment were randomly positioned next to one another in two rows over two metal shelves.

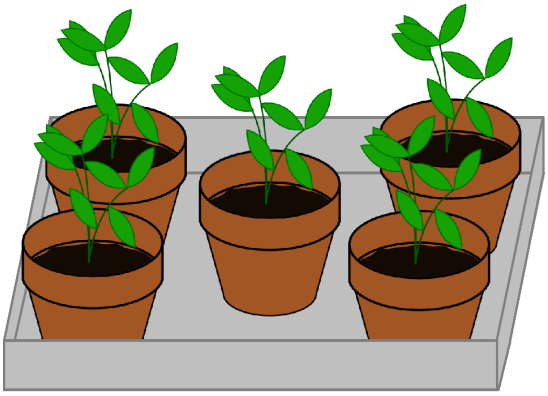

Figure 1. Arrangement of pots inside tray.

Basil seeds were planted in seedling trays filled with John Innes seed substrate no. 1, and then transferred into the experimental pots when they were at a height of 2.5–3 cm, by washing the substrate off the roots of each plant with tap water and planting the bare rooted seedlings in each pot. The greenhouse was not temperature-controlled, but kept warm between 22 and 27 °C. The air temperature was managed by opening the window whenever it became too warm for the plants. The plants were watered in the morning. Three parallel rows of 115 cm long tubular grow lights (power: 35 w; input: AC 220–240 v and 50–60 Hz; manufacturer: Philips, Eindhoven, the Netherlands), three per shelf, were placed directly above each tray at a distance of 35 cm from the top of the trays. The grow lights were set on a 16-hr light/8-hr darkness cycle. All trays were moved counterclockwise by one tray once every four days in order to guarantee uniform light exposure throughout the experiment. All the plants were harvested by hand, and the substrate carefully removed so as not to damage the roots. The roots were washed with tap water to remove the remaining

substrate. The leaf weight and whole plant weight measured were fresh weight, and the moisture level was assumed to be the same for all plants.

2.6. Measurements and Analyses

Plant yields were measured, and soil analyses were conducted to reveal the composition of the substrate before and after fertilisation with fish effluents. The effects of fish effluent fertilisation on plant growth, health, and quality were assessed by means of vegetable tissue analysis, growth rate calculations, and the overall health and appearance of the crop. The plant yield measurements were undertaken on all plants, and the weight measurements were performed in batches per replicate. The concentration of nitrogen in leaf samples was measured using a CHN analyser (model: Thermo Scientific FlashEA 1112, Waltham, MA, USA) at the University of Greenwich, Medway Campus. The whole leaf mass from each replicate was harvested, freeze-dried, and powdered using a mortar; three samples of the powdered product from each replicate were then analysed.

The analyses of the macro- and micronutrients in the fish effluent and substrate samples were performed by NRM Labs, the UK's largest independent provider of agronomic and environmental waste analysis for land-based industries, based in Bracknell, UK. The methods are described in Appendix A.

For soil microbial analysis, three initial substrate samples were taken from each substrate type prior to the beginning of the experiment, and four samples per treatment were taken after three weeks, halfway through the experiment, and on the day the experiment ended. The vials were then frozen at −21 °C until shipped for analysis by Biome Makers (Sacramento, California). DNA extraction was performed with the DNeasy PowerLyzer PowerSoil kit from Qiagen, Venlo, the Netherlands. To characterise the bacterial, archaeal, and fungal microbial communities associated with bulk soils and rhizosphere samples, the 16S rRNA and internal transcribed spacer (ITS) marker regions were selected. The 16S primer was used for sequencing bacteria and archaea, and the ITS primer was used for sequencing fungi. Primers were removed from paired-end reads using Cutadapt 3.5, and the trimmed reads were merged with a minimum overlapping of 100 nucleotides. Next, the sequences were quality-filtered by Expected Error with a maximum value of 1.0. Because of their highly conserved length, 16S reads were subjected to an additional filtering step in order to remove extreme sequence lengths. After quality pre-processing, reads with single nucleotide differences were iteratively clustered together to form ASVs (Amplicon Sequencing Variants) using Swarm v3. De novo chimaeras and remaining singletons were subsequently removed. Finally, the ASVs were compared against Biome Makers' proprietary internal reference database of amplicons using a global alignment, with 97% identity to select the best hit; in cases of multiple best hits with identical qualities, the pipeline automatically adjusted the ASV result resolution to the nearest common ancestor of the hits' taxonomies, which can decrease it to genus or family level. The reference database of amplicons was built using internal manually curated taxonomies from the latest version available of SILVA 138.1 for 16S sequences.

2.7. Statistical Analyses

Whenever possible, the data collected were then analysed statistically using R 4.1.0 software [41]; 2-way ANOVA and Tukey HSD tests were used to analyse statistical differences between the treatments. For every value set, the data distribution was explored and histograms generated to identify the presence of any outliers and test for normality. For all the experiments, no random factors were considered, and the fixed factors were the treatments; the response was a function of the treatments. For the plant yield analyses, unless otherwise specified, each plant or plant part was measured, and the average was

used in the ANOVA model, instead of using the whole replicate unit as a random factor. The standard errors were calculated from the analysis of variance residuals.

3. Results

3.1. Plant Growth and Yield

All plants grew in height and weight (Figure 2), and only one plant died of unknown causes (in replicate IT2).

Figure 2. Complete display of the two shelves where the plants were grown, showing the individual replicates (black trays), five pots per tray, and grow lights on the last day of the experiment.

Visually, and on measurement, it was evident that the plants from treatment IT—plants grown in compost-free substrate and irrigated with tap water—were much smaller and less developed than the rest, with an average height of 15.06 cm, compared to the other treatments ranging between 23.24 cm and 25.19 cm (Figure 3).

Figure 3. Comparison of randomly selected trays, one from each treatment, showing the clear visual difference of treatment IT, top left photo, from the rest of the treatments, reflecting its significantly lower product yield.

The growth yield values, including % of nitrogen by dry weight, are shown in Table 3, while the values in boxplot form are shown in Figure 4.

Table 3. Mean values and standard deviation values (±) for growth measurements. Different letters indicate statistically significant differences between treatments ($p < 0.05$), using Tukey's Honest Significant Difference (HSD) test.

Treatment	Height (cm)	Stem Diameter (mm)	Leaf Biomass (g)	Nitrogen (%)
IT	15.06 ± 1.75 b	1.84 ± 0.13 b	7.12 ± 0.85 d	2.55 ± 0.18 c
IF	23.85 ± 2.43 a	2.39 ± 0.23 ab	23.75 ± 1.66 c	4.00 ± 0.21 b
IFS	25.19 ± 2.85 a	2.51 ± 0.35 a	28.25 ± 1.66 bc	4.44 ± 0.42 ab
CT	24.58 ± 2.83 a	2.73 ± 0.29 a	23.50 ± 1.69 c	3.01 ± 0.36 c
CF	24.56 ± 1.21 a	2.75 ± 0.36 a	31.62 ± 1.93 ab	4.40 ± 0.15 ab
CFS	23.24 ± 2.42 a	2.64 ± 0.28 a	35.75 ± 3.07 a	4.90 ± 0.26 a

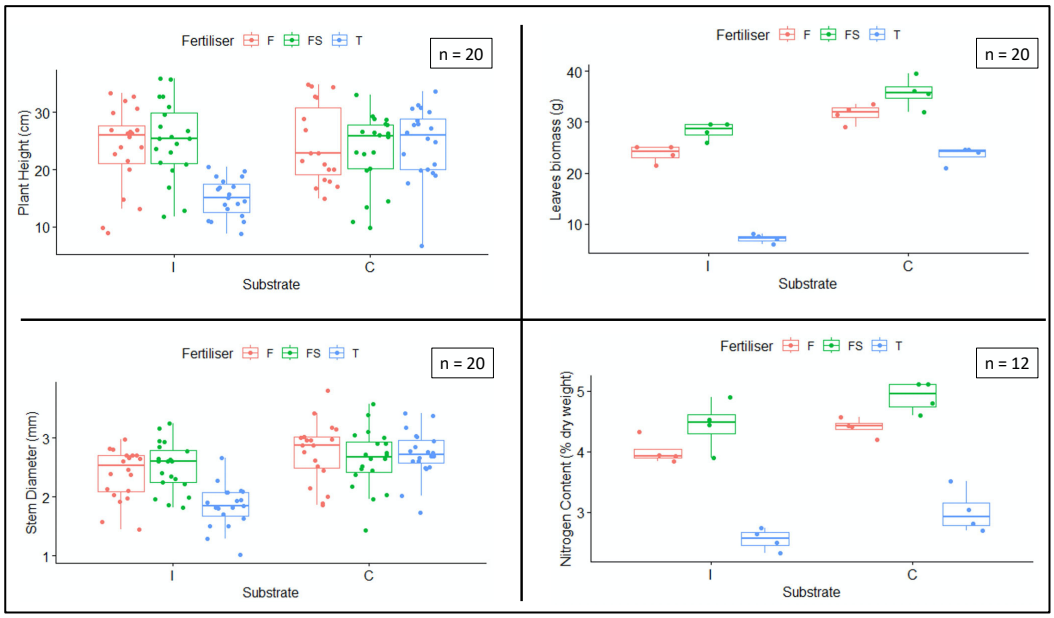

Figure 4. Boxplots for plant height (**top left**), leaf biomass (**top right**), stem diameter (**bottom left**), and nitrogen weight % (**bottom right**), with fertiliser type (F: fish water; FS: fish sludge; T: tap water) and substrate type (I: compost-free substrate; C: compost substrate). Sample sizes (n) in the top right corner of each plot.

In all measurements, the residues of the model were found to be normal, and no outliers were identified. Based on the above results, substrate, fertiliser, and the interaction between substrate and fertiliser all had a significant effect ($p < 0.05$) in all measurements undertaken, except for the nitrogen dry weight percentage, where only substrate and fertiliser had an effect, but not the interaction between them. The fish sludge treatment (FS) outperformed the fish water treatment (F) and the tap water treatment (T) for leaf biomass and nitrogen dry weight percentage in both substrate types (Table 3), with the following pattern: FS > F > T. For the leaf biomass measurement, in fact, and for the compost-free substrate, fish sludge (FS) produced an average biomass of 28.25 g, fish water (F) 23.75 g, and tap water (T) 7.12 g. For the compost substrate, the average leaf biomass was 35.75 g for fish sludge (FS), 31.62 g for fish water (F), and 23.5 g for tap water (T). For the nitrogen dry weight percentage, in the compost-free substrate, fish sludge (FS) produced an average percentage of 4.44, followed by 4.0 for fish water (F), and 2.55 for tap water (T). In the compost substrate treatment, fish sludge (FS) produced a percentage of 4.9, followed by 4.4 for fish water (F), and lastly by 3.01 for tap water (T). The same trend is observed for the stem diameter measurement of the compost-free substrate, where fish sludge (FS) produced an average diameter of 2.64 mm, followed by fish water (F) at 2.75 mm, and tap water (T) at 2.73 mm. However, in the compost substrate the fish water treatment (F) performed better than the fish sludge treatment (FS) for stem diameter (2.75 mm vs. 2.64 mm) and plant

height (24.56 mm vs. 23.24 mm). For the leaf biomass measurement, the effects of tap water and compost substrate (CT) were in a very close range to the ones from the compost-free substrate and fish water (IF), at 23.5 g vs. 23.75 average weights. Overall, the fish sludge treatment performed the best. The degrees of freedom (df), F-values, and *p*-values are reported in Table 4.

Table 4. Two-way ANOVA tables (df, F-values, and *p*-values) for the measurements, divided by substrate type, fertiliser type, and the interaction between substrate and fertiliser.

Measurement	Substrate/Fertiliser	df	F-Value	*p*-Value
Plant height	Substrate	1	5.032	0.027
	Fertiliser	2	5.973	0.003
	Substrate/Fertiliser	2	8.820	<0.001
Leaf biomass	Substrate	1	181.56	<0.001
	Fertiliser	2	162.18	<0.001
	Substrate/Fertiliser	2	13.61	<0.001
Stem diameter	Substrate	1	31.870	<0.001
	Fertiliser	2	5.454	0.005
	Substrate/Fertiliser	2	7.458	0.001
Nitrogen weight %	Substrate	1	14.959	0.001
	Fertiliser	2	98.907	<0.001
	Substrate/Fertiliser	2	0.035	0.966

Stem weights were also measured (Figure 5); however, given the lack of sufficient replicates in the measurements (the measurements were taken per treatment batch), complex statistics with reliable results were not achievable.

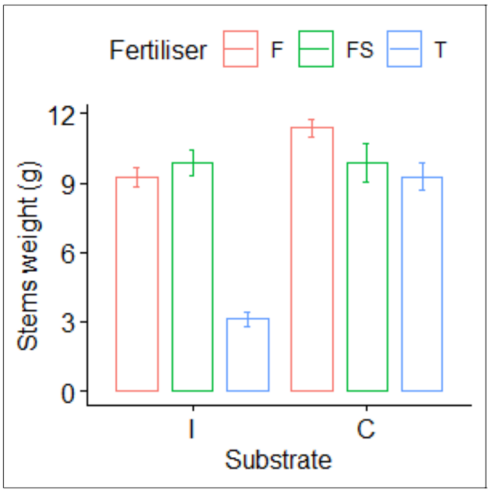

Figure 5. Stem fresh weights, arranged by substrate type (I: compost-free substrate; C: compost substrate) and fertiliser type (F: fish water; FS: fish sludge; T: tap water).

For stem weight and for all fertiliser types, compost substrate (C) performed better than compost-free substrate (I), except for the fish sludge treatment (FS), where the values were equal (9.88 g). In both substrate types, tap water (T) produced the lowest yields, at an average of 3.13 g for IT and 9.25 g for CT; however, the highest yield was produced by fish water for compost substrate (CF), with an average weight of 11.38 g, and by fish sludge for compost-free substrate (IFS), with an average weight of 9.88 g.

3.2. Soil Microbiome

In all treatments, the final species count was higher than the initial one; this was also true for the IT treatment, where only tap water was used (Figure 6).

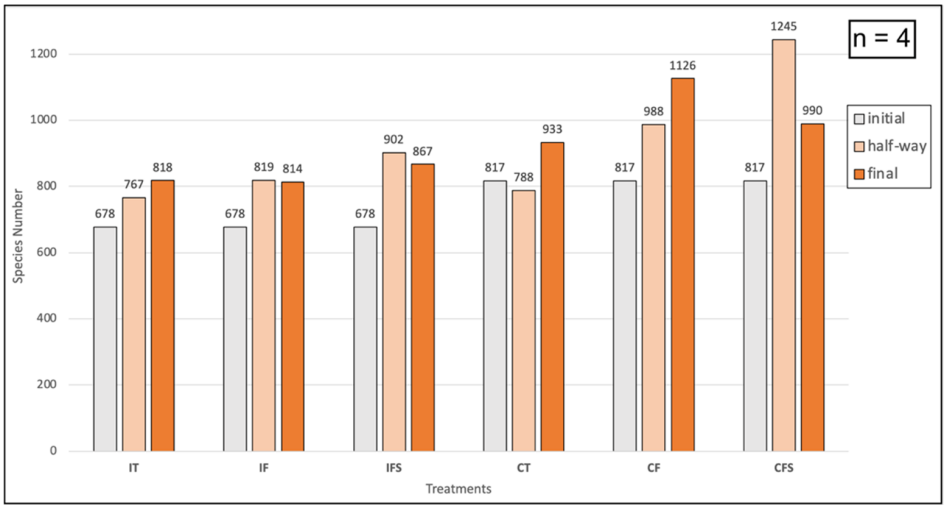

Figure 6. Total species (bacteria, archaea, and fungi) diversity across treatments at initial, halfway, and final experimental stages, with relative species number. The same initial compost-free substrate was used for treatments IT, IF and IFS, and the same initial compost substrate was used for treatments CT, CF, and CFS. Sample size (n) in the top right corner.

In treatments IF, IFS, and CFS, the species count was higher (819, 902, and 1245, respectively) halfway through the experiment than at the end (814, 867, and 990, respectively). The initial compost-free substrate, used in treatments IT, IF, and IFS, was lower in species count than the compost substrate used in treatments CT, CF, and CFS (678 vs. 817). This difference was also reflected in the final species count, given that all final species counts from treatments CT, CF, and CFS (933, 1126, and 990, respectively) were higher than all species counts from treatments IT, IF, and IFS (818, 814, and 867, respectively). The shared species number between the two initial substrates was 372 species.

The most prevalent archaeal and bacterial (Figure 7) and fungal (Figure 8) genera across treatments in halfway and final samples are displayed.

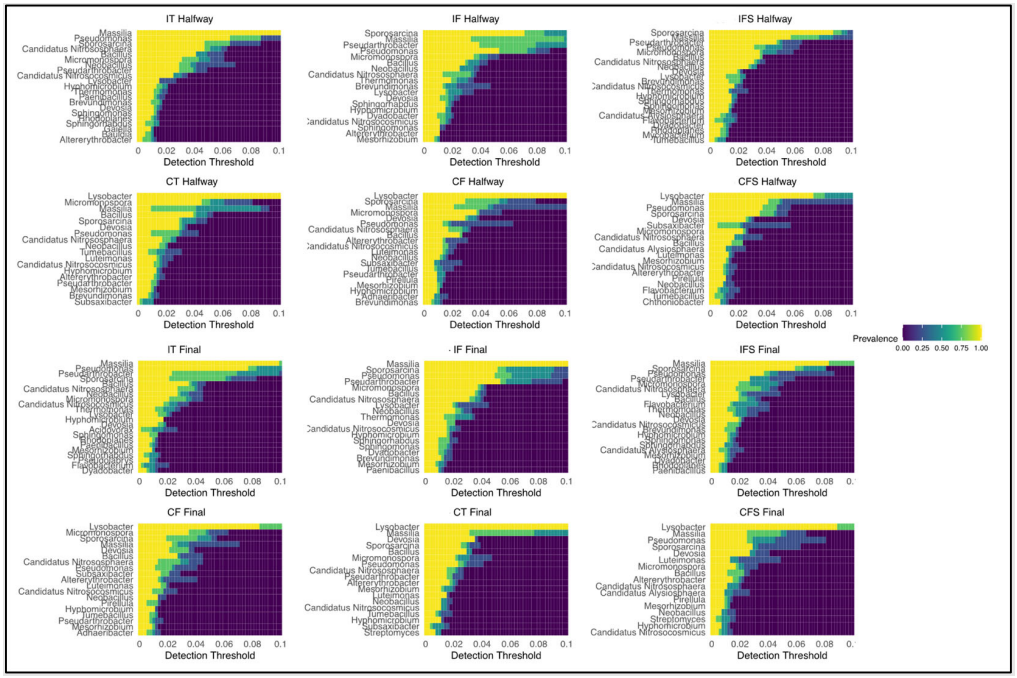

Figure 7. Heatmaps of the most prevalent bacterial and archaeal genera both in the middle (**top two rows**) and at the end (**bottom two rows**) of the experiment, across all treatments.

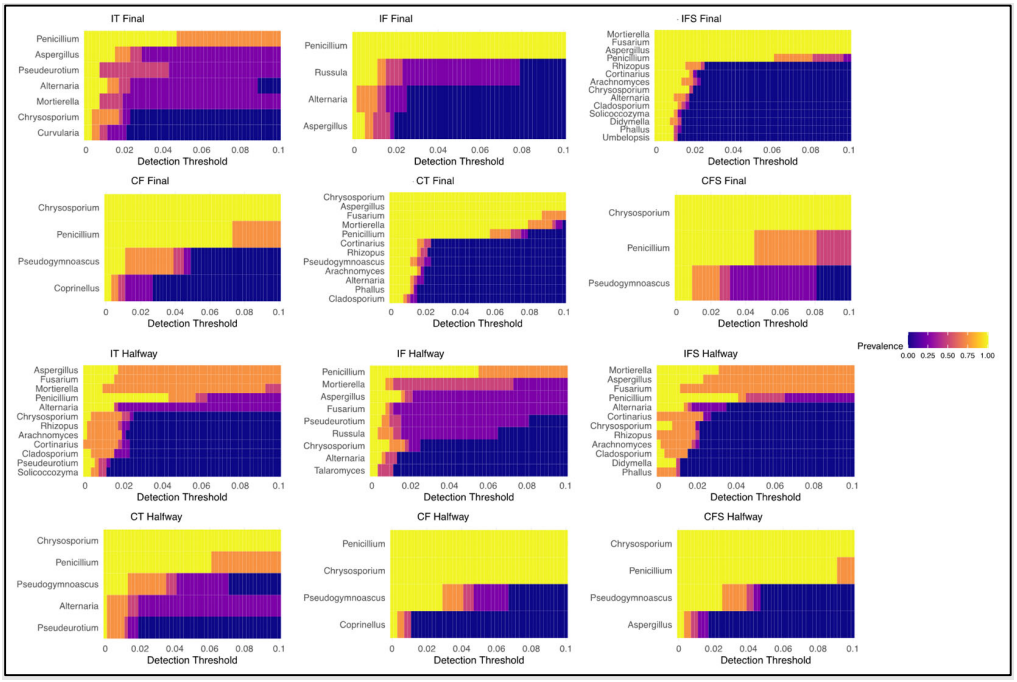

Figure 8. Heatmaps of the most prevalent fungal genera both in the middle (**top two rows**) and at the end (**bottom two rows**) of the experiment, across all treatments.

Regarding bacterial and archaeal genera across treatments, and taking into account the six most prevalent genera, *Massilia* and *Sporosarcina* were present in all treatments, in both halfway and final samples. In halfway samples, *Micromonospora* was present only in treatments with compost-free substrate (IT, IF, and IFS), and *Lysobacter* and *Devosia* were present only in samples with compost substrate (CT, CF, and CFS). In final samples, *Pseudarthrobacter* was only present in treatments with compost-free substrate (IT, IF, and IFS), and *Lysobacter* was only present in samples with compost substrate (CT, CF, and CFS). Regarding fungal genera across treatments, and taking into account the six most prevalent genera, *Penicillium* was present in all treatments, in both halfway and final samples. In halfway samples, *Fusarium* was present only in treatments with compost-free substrate (IT, IF, and IFS), and *Pseudogymnoascus* was present only in samples with compost substrate (CT, CF, and CFS). In both bacterial and archaeal, and fungal analyses, most genera were found across multiple treatments and, in general, little variation was found, including when different fertiliser sources were considered.

4. Discussion

The yield results showed that the presence of compost and fish effluents satisfied the nutrient requirements of the plants, whilst the plants that were grown in a compost-free substrate and watered with tap water (treatment IT) did not have their nutritional requirements met. The results also indicated that fish effluents fully substituted for compost in terms of the nutrition that the plants needed for leaf biomass. The significant effect of combining compost and fertilisation with the fish effluents on the yield of the plants was confirmed by the 2-way ANOVA analysis (Figures 4 and 5). In fact, with the sole exception of nitrogen dry weight percentage, the variables substrate, fertiliser, and the interaction between the two all had a significant effect ($p < 0.05$) in all measurements undertaken. In most cases fish sludge produced the highest yields, reflecting its higher nutrient concentration which was also demonstrated by a previous study on onions [42]. In the case of total fresh weight, compost substrate enhanced the yield, and fish effluents had a positive effect, within each substrate type, with fish water resulting in an almost

three-fold increase in weight compared with tap water in compost-free substrate (average weight for IT was 10.25 g vs. 33 g for IF), similar to the influence of compost (average weight for CT was 32.8 g). Total weight and leaf weight both follow the same pattern, from highest to lowest yield: CFS, CF, IFS, IF, CT, and IT. For leaf weight and nitrogen dry weight percentage, the presence of fish water and sludge significantly ($p < 0.05$) enhanced the weight and nitrogen percentage for both compost and compost-free substrate; for height, this was only true for compost-free substrate. IT being last accords with what was observed for height, leaf biomass, and stem diameter; however, CFS and CF being first indicates that for this experiment, there is an additive effect of compost and fish effluents, with fish sludge performing better than fish water. This is to be expected, as fish sludge is much higher in all nutrients analysed (Table 1), with a 57% increase in nitrate content compared with fish water (44.3 mg/L vs. 69.6 mg/L). This is also reflected in the next two treatments that performed the best in terms of weight, IFS and IF; in these cases, fish sludge also performed better than fish water (average weight 38.125 g vs. 33 g). Second to last is treatment CT, where plants were grown in compost substrate and only watered with tap water; this indicates that fish water and fish sludge performed better than compost, in both total plant weight and total leaf weight.

The effects of fish effluents applied to soil for basil cultivation have only been investigated in two studies. Omeir, M.K. et al. [43] found that irrigation with fish farm effluent significantly increased the growth rates and nutrient content of the plants, compared to river water irrigation. Similarly, Valkovszki, N.J. et al. [44] reported that irrigation with fish farm effluents significantly increased the fresh and dry weight of shoots and roots, leaf number, and stem height in basil, indicating positive effects on plant growth.

The effect of fish effluent irrigation has also been investigated on another herb, oregano. Kimera, F. et al. [45] investigated the growth and essential oil content of *Origanum syriacum*. The results demonstrated that fish effluent irrigation significantly improved plant growth, with the treatment reaching maximum plant height and highest fresh and dry herbage yield. Although not performed in greenhouse conditions, these results are consistent with the results of this study, where the plants not fertilised with fish effluents (CT treatment) grew significantly less than the ones that were fertilised with fish effluents. No study so far, however, has compared the performance of compost and fish effluents for growing basil. Regarding the concentration of nitrogen in the leaves, fish sludge outperformed fish water in both substrate types, following the trend found for the leaf biomass, and reflected in the total plant fresh weight.

These results overall, and specifically the total fresh weight, are consistent with the literature on the positive effects of combining compost with fertiliser use. A study demonstrated that the combination of biochar compost and inorganic nitrogen fertiliser improved nitrogen uptake in rice plants [46]. Similarly, it was found that combining compost with organic fertiliser application in organic systems increased nutrient availability to plants in the early stages [47]. Furthermore, the combination of compost and nitrogen fertiliser improved yields, yield components, and nitrogen uptake in wheat cultivars [48]. These findings collectively support the notion that the combination of compost and fertiliser can enhance nitrogen uptake and concentration in plants. Moreover, a study revealed that the highest values for yield and its components were obtained by humic acid under the highest level of nitrogen fertiliser and compost treatment [49]. This suggests that the combination of compost and nitrogen fertiliser not only enhances nitrogen concentration, but also positively influences overall plant yield and components.

Regarding the microbiome communities found in the substrates, the final species count was higher at the end of the experiment than at the beginning in all treatments, including the ones where only tap water was supplied. This is probably due to the John

Innes seed substrate no. 1, where the seedlings were grown prior to being transferred to the pots. In fact, the measured initial diversity came from the manufactured substrates, which did not take into account the possible inoculation of further species derived from the tap water, air, or the seedling substrate where the basil seeds germinated. It is therefore likely that the remaining substrate surrounding the roots of the seedlings as they were being transferred into the experimental pots contributed to the overall microbial diversity, despite most of the substrate having been washed off. On the other hand, the soil microbiome has been extensively shown to mutate as plant growth progresses, its biodiversity having significant effects on the success and function of plant-associated microbiomes; thus, the increase in species diversity could have also occurred naturally alongside plant growth and development [50]. Compost has been documented as having rich microbial communities. Rastogi, M. et al. [51], for example, highlighted the role of microbes in solid waste composting, emphasising their vital contribution to the decomposition of organic matter and the production of soil-enriching compost. These microbes can, via compost, be introduced into the potting substrate. Green, S.J. et al. [52] investigated the succession of bacterial communities during early plant development and the transition from seed to root, emphasising the introduction of high numbers of microbial cells into soils or potting substrates through compost amendments. Michel, F.C. et al. [53] demonstrated the inoculation of compost-amended potting mixes with biocontrol agents and other microbes to induce systemic disease resistance in plants, indicating the potential for compost-derived microbes to influence soil and plant health. Furthermore, Zhao, J. et al. [54] screened and applied microbial inoculants for sewage sludge composting, highlighting the importance of understanding and utilising specific microbial communities in the composting process. Overall, the literature provides substantial evidence supporting the potential for microbes from compost to inoculate potting soil, influencing soil properties, plant growth, and overall soil health.

For treatments IF, IFS and CFS, the species count was higher halfway through the experiment than at the end, the difference being the lowest (5 more species) for IF, higher for IFS (35 more species), and highest for CFS (255 more species). This could be due to some initial measured increase in diversity derived from the seedling substrate, which then stabilised and decreased. The initial compost substrate was higher in diversity than the initial compost-free substrate (139 more species, or a 20.47% increase), most likely because of the enhancing effect of composted green material. This initial higher diversity in the compost substrate likely resulted in a higher microbial diversity in the treatments with compost substrate (CT, CF, CFS) than in the treatments with compost-free substrate (IT, IF, IFS); indeed, as the nutrients already present in the substrate combined with the nutrients provided by the fish effluents, in the same way, the microbial diversity supplied by the compost was enhanced by the supplementation of fish effluents. It has been found that potting soil mixed with compost can indeed lead to higher microbial diversity. The authors of [55] highlighted that composting leads to changes in bacterial and fungal communities, indicating an increase in microbial diversity. Neher, D.A. et al. [56] reported that compost can effectively replace peat-based potting media without negatively affecting plant growth, indicating the potential for compost to enhance microbial diversity in potting mixes. These findings are consistent with the study by [57], which shows that compost-based growing media can improve soil microbial activity. The introduction of compost into potting mixes was shown to enhance microbial diversity, as evidenced by the changes in bacterial and fungal communities, thus supporting the findings of this study.

Massilia, a major group of rhizosphere- and root-colonising bacteria [58], was present in the six most prevalent genera across all treatments. *Micromonospora*, a group of aerobic, mycelium-forming bacteria [59], was only present within the six most prevalent genera in

the compost-free substrate treatments (IT, IF, and IFS), and *Lysobacter* and *Devosia* only in the treatments with compost substrate (CT, CF, and CFS). *Lysobacter* has been observed on the surface of fish tanks of aquaponic systems [60], as well as in RAS biofilter samples of aquaponic systems [61]; this genus has been identified as a plant growth-promoting bacterium (PGPB) that protects plants from disease through the production of antibiotics [62]. *Devosia* contains some potential nitrogen-fixing bacteria, and the genus has been found to grow on lettuce (*Lactuca sativa*) roots in aquaponic systems [60], as well as in all biofilters and sumps of an aquaponic system [61]. Taking only the most prevalent six genera into account per treatment, specific genera were only observed in treatments with compost substrate, and others only in treatments with compost-free substrate. This distinction is likely to have been caused by the initial substrate composition and the microbial communities associated with each. From the genera found, there does not seem to be a pattern associated with any specific treatment. In fact, in both bacterial and archaeal, and fungal analyses, most genera were found across multiple treatments. The impacts of fish effluents on the soil microbiome have not been extensively studied, especially not in greenhouse conditions. Some studies have however found that irrigation with fish effluents changes the structure of soil microbial communities. Chen, L. et al. [63] showed that aquaculture wastewater irrigation can change soil microbial functional diversity and community structure in arid regions, highlighting the potential impact of fish water irrigation on soil microbiota. Guan, W. et al. [64] found that the soil bacterial communities in rice fields irrigated with aquaculture wastewater can be impacted by the intestinal bacteria of the fish. Irrigation with fish-processing effluents has also been found to increase the nitrification rate and abundance of ammonia-oxidising archaea and bacteria in arid soils, but not to affect bacterial amoA genes [65]. Fish-processing effluent discharges in arid soils in Patagonia have also been shown to influence physicochemical properties and prokaryotic community structure, potentially benefitting native salt-tolerant plant irrigation [66]. However, Sun, Y. et al. [67] found that irrigation using activated brackish water improved soil fertility, which may have conferred dominant microbial populations with a strong ability to resist external interference to the soil environment, resulting in no significant impact on soil bacterial diversity. Given the limited knowledge of each particular microbial genus, it is difficult to assess the changes in the structure of the community, and how significant they were.

5. Conclusions

This experiment indicated the great potential of using fish water and fish sludge as fertiliser for greenhouse-based herb production. Fish effluents were able to fully substitute for compost, and to perform better than compost in some parameters, such as plant and leaf weight, thus showing that compost could be fully or partially substituted with fish effluents. The study therefore contributes valuable insights into the potential of fish effluents as a viable alternative to traditional compost in greenhouse agriculture. Whilst not without the need for further investigation, the results suggest a more sustainable approach to nutrient recycling and waste reduction in agricultural practices. The increase in microbe species count in all treatments, including IT where only tap water was used, was likely caused by the inoculation of microbiome species coming from the substrate where the seedlings were grown; inoculation could additionally have come from the tap water or ambient air, as the experiment was not performed in a sterile environment. The higher species diversity associated with the compost substrate treatments likely influenced the final diversity, given that a higher diversity was observed in the final samples of the compost substrate treatments than in the final samples of the compost-free substrate treatments.

In conclusion, this experiment aligns with existing literature by demonstrating that the presence of compost in growing media enhances microbial diversity. Regarding the different

genera observed across treatments, for both bacteria and archaea, and for fungi, most genera were found across multiple treatments, without a clear genus pattern influenced by treatment. Given the higher microbial diversity observed following the application of the fish effluents, this experiment showed the potential for using fish effluents to create a 'living soil', which is one of the prerequisites for organic certification in Regulation (EU) 2018/848 (Annex II, rule 1.1) [1]. This study underscores the need for additional research to substantiate the implications of compost application, particularly in conjunction with fish effluent fertilisation, on soil microbial genera. Whilst the current findings suggest a positive influence on microbial diversity, a more comprehensive understanding of the nuanced dynamics necessitates further exploration of the intricate interactions between compost, fish effluent fertilisation, and microbial communities, elucidating the specific taxonomic composition and functional roles of microbial genera in response to these soil management practices. The conclusions of this study are based on a single growing cycle, which may not fully account for the long-term effects of repeated fish effluent application. Because organic fertilisers such as fish effluents can gradually shape soil fertility and microbial communities over multiple seasons, further research should include extended or multi-year studies. Such an approach would provide a more comprehensive evaluation of how fish effluents influence crop performance, soil quality, and ecological sustainability over time, thereby offering stronger evidence for their potential inclusion in organic production systems. Future research should also focus on understanding the best possible combination of fertilisation frequency, amount of fertiliser use, and type (filtered vs. unfiltered fish effluents), and investigating how much compost the effluents can truly substitute without compromising crop yield.

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Appendix A

The following are the methods employed by NRM Labs to analyse the substrate and fish effluent samples.

Substrate—ammonium nitrate extractable calcium and sodium

The samples were air-dried at a temperature not exceeding 30 °C and sieved through a 2 mm mesh. The calcium and sodium were extracted by shaking the samples with M ammonium nitrate at 20 °C for 30 min. After filtration, the calcium concentration in the extract was measured by Atomic Absorption Spectrophotometry.

Substrate—available sulphate

The samples were air-dried at a temperature not exceeding 30 °C and sieved through a 2 mm mesh. The available sulphate was extracted under controlled conditions using a phosphate buffer (ratio 1:2). The filtered extract was then analysed by Inductively Coupled Plasma Emission Spectroscopy.

Substrate—DTPA-extractable manganese, iron, copper and zinc

The samples were air-dried at a temperature not exceeding 30 °C and sieved through a 2 mm mesh. Zinc, manganese, iron, and copper were extracted at 20 °C using a DTPA solution in a 1:2 ratio. In principle, the DTPA extraction allowed the metal in the samples to reach equilibrium with the chelating agent. A pH of 7.3 enabled DTPA to extract iron as well as other metals.

Substrate—hot water-soluble boron

The samples were air-dried at a temperature not exceeding 30 °C and sieved through a 2 mm mesh. Boron availability was assessed by hot water extraction. The boron concentration in the extract was measured using ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy).

Substrate—organic matter content by loss on ignition at 430 °C

The samples were air-dried at a temperature not exceeding 30 °C and sieved through a 2 mm mesh. The organic matter was destroyed by dry combustion at 430 °C, and the loss in weight of the samples was reported as the organic matter content (in %) on a dry matter basis.

Substrate—mineral nitrogen (available N)

The samples were chopped and mixed to achieve a homogeneous sample. A portion was shaken with 2 M KCl to extract the mineral-N fractions, and a dry matter determination was performed. If mineralisable N was required, a second portion was incubated anaerobically at 40 °C in water for one week to mineralise the nitrogen, followed by extraction of the mineralised fraction with KCl. Once in solution, nitrate-N, nitrite-N, and ammonium-N were measured colourimetrically. Nitrate-N and nitrite-N were determined based on the formation of a diazo compound between nitrite and sulphanilamide, which was then coupled with N-1-naphthylethylenediamine dihydrochloride to form a red azo dye. The colour was measured at 540 nm. In channel one, nitrate was fully reduced to nitrite by cadmium metal in an open tubular cadmium reactor (OTCR), so total oxidised nitrogen (TON) was measured as the sum of nitrite plus reduced nitrate. In channel two, only nitrite was measured. Nitrate-N was calculated by subtracting the nitrite value from the TON. For ammonium-N, in channel three, ammonium reacted with alkaline hypochlorite and phenol to produce indophenol blue. Sodium nitroprusside served as a catalyst in the formation of indophenol blue, which was measured at 640 nm. Precipitation of calcium and magnesium hydroxides was prevented by adding a combined potassium sodium tartrate/sodium citrate complexing reagent.

Substrate—pH and lime requirement

The samples were air-dried at a temperature not exceeding 30 °C and sieved through a 2 mm mesh. pH was defined as the pH, measured potentiometrically, of a suspension created by stirring the samples with water in a 1:2.5 ratio. As temperature influenced pH measurement, the measurement was performed in a temperature-controlled setting.

Substrate—Olsen's extractable phosphorus

The samples were air-dried at a temperature not exceeding 30 °C and sieved through a 2 mm mesh. The available phosphorus was extracted at 20 °C by shaking the samples with 0.5 M sodium bicarbonate for 30 min. The phosphorus concentration was then determined by flow injection analysis/colourimetry, whereby acid ammonium molybdate formed the phosphomolybdate ion. This ion, when reduced with ascorbic acid, created a blue complex, measured spectrophotometrically at 880 nm. Calibration was carried out with commercial phosphate standards traceable to SI units.

Substrate—ammonium nitrate-extractable potassium and magnesium

Potassium and magnesium were extracted at 20 °C by shaking the samples with 1 M ammonium nitrate for 30 min. After filtration, the concentrations of potassium and magnesium in the extract were determined by Atomic Absorption Spectrometry, calibrated with commercial standards traceable to SI units.

Fish effluent—nitrate nitrogen

Nitrate-N was measured colourimetrically based on the formation of a diazo compound between nitrite and sulphanilamide, which was subsequently coupled with N-1-naphthylethylenediamine dihydrochloride to form a red azo dye, measured at 540 nm. In channel one, nitrate was fully reduced to nitrite by cadmium metal in an open tubular cadmium reactor (OTCR). Thus, total oxidised nitrogen (TON) was measured as the combination of nitrite plus reduced nitrate. In channel two, nitrite alone was measured. Nitrate-N was calculated by subtracting nitrite from the TON.

Fish effluent—dissolved elements

The samples were filtered to remove particulate matter. The elements in the filtrate were then measured either by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) or by Inductively Coupled Plasma Emission Spectroscopy (ICP-OES), depending on the specific element of interest and the required Limit of Detection (LOD). ICP-MS enabled lower LODs.

Fish effluent—electrical conductivity

The specific conductivity of the solution was determined using an EC meter, and the measurement was standardised to 25 °C.

Fish effluent—pH

The pH of the solution was measured potentiometrically. As temperature influenced the result, the measurement was performed in a temperature-controlled environment.

Fish effluent—total dissolved solids

After filtration through glass fibre paper to remove suspended solids, a known volume was dried at 180 °C and the residue weighed in order to calculate the total dissolved solids.

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