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Utilisation of *Miscanthus x giganteus* L. Based C-Rich Fertilisers for N Immobilisation and Microbial Biomass Build-Up in a Crop Rotation

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Abstract: Cultivation of perennials such as *Miscanthus x giganteus* Greef et Deuter (*Mis*) combines the provision of ecosystem services and the generation of additional carbon sources for farming. The potential of *Mis* based fertilisers, regarding immobilisation of inorganic nitrogen (N) and build-up of soil organic matter (SOM), was tested in a field trial. Therefore, a crop rotation of winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris* L.) and winter wheat (*Triticum aestivum* L.) was set up. The tested treatments were a mixture of Cattle Slurry (CS) and *Mis*, a mixture of CS and Wheat Straw (CS–WS), Cattle Manure (CM) from *Mis* shredded bedding, CM from WS shredded bedding, a pure CS, Urea Ammonium Nitrate (UAN) and a treatment without any N applied (NoN). When the carbon-rich fertilisers (both mixtures and manures) were applied to cereals, they led to a slight N immobilisation compared to pure CS, whereas differences were mostly not significant. Furthermore, *Mis* fertilisers were at least as efficient as WS-based organic fertilisers in inducing a contribution of SOM build-up and in reducing inorganic N before winter and thus preventing N losses, whereas differences were mostly not significant.

Keywords: *Miscanthus x giganteus*; *Miscanthus* bedding; fertiliser from *Miscanthus*; N immobilisation; C source; *Miscanthus*-carbon; microbial biomass C; microbial biomass N; soil organic matter; C sequestration

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1. Introduction

In the last decades, technological developments, agricultural subsidies and the world market trade have facilitated an increase in animal husbandry and bioenergy production, as well as the access to mineral nitrogen (N) fertilisers [1–3]. This has changed production methods and contributed to specialisation of agricultural production and an intensification of agricultural land use. However, although this has improved the availability of food, access to mineral nitrogen may also threaten the sustainability of agricultural production in the long term [1,3]. Unsustainable soil management in arable farming can lead to soil degradation and consequently to negative effects on crop production. This may often be compensated by an increased fertilisation, but high N input often leads to N losses in the form of nitrate leaching into ground and surface waters, resulting in eutrophication [4]. Furthermore, due to enhanced N inputs, the risk of ammonia (NH_3) emissions with toxic effects on the respiratory system of mammals and humans and nitrous oxide (N_2O) emissions, which is a potent greenhouse gas, has increased [5–8].

In addition, past land-use changes by conversion of grassland to cropland and changed production practices, such as the replacement of cereals with root crops and fodder crops with lower carbon/nitrogen ratio (C/N ratio), all led to a decrease in soil organic carbon (SOC) in many cases [9–13]. Consequently, soils become more vulnerable to extreme weather conditions, including periods of drought, heavy rainfall and strong winds, which can result in soil erosion and yield losses. Compared to annual crops, the cultivation of perennial crops such as *Miscanthus* (*Mis*) has numerous ecological advantages. *Miscanthus x giganteus* L., a sterile hybrid of *Miscanthus x sacchariflorus* (Maxim.) Hack. and *Miscanthus x sinensis* Andersson [14,15], can protect soil against erosion and can cause an accumulation of over one t C ha^{-1} $year^{-1}$ in soil [16]. In contrast, soil organic matter (SOM) contents provide a greater level of resilience for plants and soil microbial biomass (MB), contributing to stable yield and quality parameters.

Furthermore, it is essential to use organic fertilisers and other C sources in a way that retains N and C in the crop–livestock–soil system and stops further SOC reduction or that even promotes further SOC storage. Soil microorganisms have a key function because they regulate essential C and N turnover processes in the soil. Nutrient mobilisation processes are induced by enzymatic activity and nutrient immobilisation is caused by microbial uptake of nutrients; both activities are closely related to the size of the soil MB [17,18]. The MB, which is dominated by fungi and bacteria [19], fulfils important functions in the soil. Anabolic processes lead to the incorporation of C and nutrients into biological structures and catabolic processes lead to mineralisation of organic N to NH_4^+ and of organic C to carbon dioxide (CO_2) [20]. When organic fertilisers are applied, not all of the N supplied gets mineralised in the year of application. A part of the N remains in the soil, and some becomes plant available in the following years, through microbial mineralisation processes, depending on the environmental conditions, tillage intensity and the specific characteristics of the applied organics [21–23].

If microbial nutrient mineralisation from organic fertilisers does not occur simultaneously with plant N demand and uptake, either N deficiency or residual nitrate leaching can occur. Especially at the end of the vegetation period, inorganic N may be transferred into soil depths with the onset of the autumn rains and cannot be reached by plant roots anymore. Leaching mainly occurs as nitrate (NO_3^-), due to its monovalent negative charged anion. In contrast, NH_4^+ is positively charged and mostly adsorbed at negatively charged colloidal surfaces, avoiding the risk of leaching. Therefore, N fertilisation tailored to crop demand is essential to avoid N surplus and to minimise negative environmental effects. Insufficient N fertilisation fundamentally reduces yield and quality of the crops, thus reducing marketing opportunities and revenue. Therefore, knowledge on the N dynamics of organic fertilisers applied before planting is essential and needs to be taken into account in the farmers' N fertilisation strategy. In Europe, inappropriate N fertilisation has led to concentrations of nitrate in groundwater bodies in some regions exceeding the EU-limit of 50 mg L^{-1} [24]. For the reduction of nitrate concentrations below the critical value, regulations on fertiliser application have been tightened in some European countries. The cultivation of catch crops can immobilise inorganic N in plant biomass and, when N is abundantly available, the C rhizodeposition of catch crop stimulates microbial N immobilisation [25–27].

It is well known that incorporation of C-rich components such as cereal straw stimulates anabolic processes in soil microorganisms and consequently reduces N losses, as well as contributing to SOC maintenance [28–33]. In greenhouse experiments we already showed that *Mis* biomass provides a suitable C source to induce N immobilisation in soil; part of this N immobilisation is caused by microbial growth which also contributes to C sequestration [34]. The cultivation of C-rich crops such as annual cereals can be supplemented with perennials such as *Mis*, whereas *Mis* provides important ecosystem services and can create new sales and utilisation opportunities [35]. *Mis* is certified as a greening crop in Germany (a crop subsidised for its ecological value) [36]. It can be cultivated on marginal sites where the cultivation of other crops is economically not feasible and can be cultivated as a low-input crop because of low fertiliser demand and no need for weed control [37]. Perennial crops such as *Mis* can protect soil against erosion. The harvest of *Mis* usually takes place in spring, immediately before new shoots of *Mis* appear. Consequently, the tight *Mis* habitat provides opportunities for rare wildlife to protect themselves from predators and weather conditions during the winter months and increases structural diversity in open agricultural landscapes. The specific cultivation can contribute to the restoration of biotope cross-linking [38,39], which has been lost in many cases by the structural change in agriculture in recent decades [40–43]. *Mis* biomass can be used as feedstock in anaerobic digestion [44,45], growing media in soilless cultivation [46], can be cascaded to livestock farms in the form of bedding material [47] or it is used as an additive for the packaging industry or as construction material [35]. However, the question is whether C input in the form of *Mis* can be applied as a straw substitute (e.g., because the cultivation of *Mis* has numerous ecological benefits or because cereal straw is exported) for microbial N immobilisation and its effects on nitrate leaching and crop yields. Therefore, in a field trial, a crop rotation with winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris* L.) and winter wheat (*Triticum aestivum* L.) was set up to test the effects of two novel N-containing and C-rich organic farm fertilisers based on *Mis*.

In this context, our hypotheses were (i) *Mis* is as effective as Wheat Straw in immobilising additional inorganic N from mineralisation of slurry or manure and thus reduces nitrate leaching as effective as Wheat Straw; (ii) *Mis* and Wheat Straw are identical in affecting yield and quality parameters of crops of a crop rotation; (iii) microbial biomass make use of *Mis* as a C source for biomass build-up and thus contribute to C sequestration.

2. Materials and Methods

2.1. Site Description

A field trial was set up at Campus Klein-Altendorf (University of Bonn, Rheinbach, Germany) from September 2017 to August 2020. A typical crop rotation for the Rhine region was chosen, this consisted of winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris*) and winter wheat (*Triticum aestivum* L.). The set-up was chosen to compare the N dynamics of two organic fertilisers based on *Miscanthus x giganteus* L. (*Mis*) in an arable soil of a conventionally farmed agricultural site. Average annual precipitation for 2018 and 2019 (420 and 490 mm) was lower than the long-term average (2007 to 2016) of 633 mm. Average annual temperature for 2018 and 2019 (11.0 and 11.5 °C) was greater than the long-term average (2007 to 2016) of 10.2 °C.

The experiment was carried out on a Gley-Cambisol. The previous, unfertilised grassland was converted to arable in 2013. As determined by particle-size analysis according to DIN ISO 11277:2002-08 [48], the soil texture is a silty loam (Table 1) and the location of the field is 50°36′3″ N, 07°01′37″ E; WGS 84. The basic soil properties such as pH [49], P_2O_5, K_2O [50], Mg [51], B, Cu, Mn, Fe [52], SOM [53] and N_t [54] are given in Table 1.

Table 1. Contents and amounts of basic soil properties of the experimental site for the top 30 cm. Values show means and standard deviation (*n* = 5; for SOM, SOC, N_t, C/N: *n* = 6).

pH (H_2O)	**P (mg kg^{-1})**	**K (mg kg^{-1})**	**Mg (mg kg^{-1})**
6.3 ± 0.06	11.4 ± 2.7	10.4 ± 1.6	14 ± 1.9
B (mg kg^{-1})	**Cu (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Fe (mg kg^{-1})**
0.5 ± 0.04	6.3 ± 0.5	169.4 ± 47.4	196.3 ± 18.6
SOM (%)	**SOC (%)**	**N_t (%)**	**C/N (ratio)**
3.9 ± 0.7	2.3 ± 0.4	0.27 ± 0.02	8.5 ± 1.2
Clay (g kg^{-1})	**Silt (g kg^{-1})**	**Sand (g kg^{-1})**	
229	597	173	

SOM = soil organic matter; SOC = soil organic carbon; N_t = total N; C/N = carbon/nitrogen ratio.

The biomass of *Mis* grown on another field was used for two utilisation pathways; first mixed with Cattle Slurry (CS) to create a Cattle Slurry–*Miscanthus* mixture (CS–*Mis*) and second used as bedding material creating Cattle Manure based on *Mis* (CM–*Mis*). For comparison with *Mis*, Wheat Straw (WS; *Triticum aestivum* L.) biomass was used to create a Cattle Slurry–Wheat Straw mixture (CS–WS) and Cattle Manure based on WS (CM–WS).

The *Mis* biomass was harvested in April 2017 (used for organic N fertilisation of winter barley), in April 2018 (used for organic N fertilisation of mustard and sugar beet) and in April 2019 (used for organic N fertilisation of winter wheat), respectively, with a forage harvester (Krone Big X 480, Krone, Spelle, Germany) with a set cutting length of 30 mm. WS biomass, used for mixing with CS, was broken up and baled by a Claas Quadrant 3200 FC big baler with a ROTO CUT front chopper and FineCut cutting unit (Claas, Harsewinkel, Germany). WS biomass, used for comparison to *Mis* as bedding, was not chopped and cut. WS biomass was harvested in August 2017 (used for organic N fertilisation of winter barley and mustard), in August 2018 (used for organic N fertilisation of sugar beet) and in August 2019 (used for organic N fertilisation of winter wheat). As a reference treatment for the two mixtures, a pure CS was tested. For the determination of the best possible mixing ratio of both mixed treatments (CS–*Mis*, CS–WS) concerning maximum absorption of CS to *Mis* and of CS to WS biomass, different amounts of CS (from one to ten kg of CS in steps of 0.5 kg CS) were mixed with one kg of *Mis* or WS. A complete absorption was achieved by soaking the biomass for seven days. The final mixing mass ratios were five to one for CS to *Mis* and 8.5 to 1 for CS to WS. After mixing, the two mixture treatments were stored for five weeks on a manure slab and covered with a silage film to prevent precipitation intrusion and allow for N immobilisation. The other option to use *Mis* on a farm was the use of *Mis* as bedding material in livestock. For this purpose, cattle were bedded with *Mis* (Cattle Manure from *Miscanthus* = CM–*Mis*) and, as a reference, cattle were bedded with WS (Cattle Manure from Wheat Straw = CM–WS) according to standard farm practice and mucked out after about six weeks. In addition, two further treatments were tested, this was a mineral N fertilisation (Urea Ammonium Nitrate solution = UAN) as well as a treatment without any N applied (no nitrogen applied = NoN).

The application dates and rates of the N fertilisers used in the field trial are listed in Figure 1. The nutrient content of the applied fertilisers was determined by a certified laboratory and is listed in Table 2. Based on the nutrient analyses of each fertiliser, the amount of applied fertilisers was calculated, to ensure that the same amount of N across all treatments could be applied, except for NoN. The solid fertilisers were applied by manure forks and the liquid fertiliser by watering cans. The crops were grown conventionally and the straw of winter barley and mustard was removed. Before the start of the field experiment, winter barley (2016/2017) and winter wheat (2015/2016) were cultivated and straw was removed for both. The trial was set up as a randomised block design with four replicates per treatment. Each plot had a size of 6 m × 16 m, with a sampled area of 3 m × 8 m (to ensure uniformity of incorporation of the organic fertilisers).

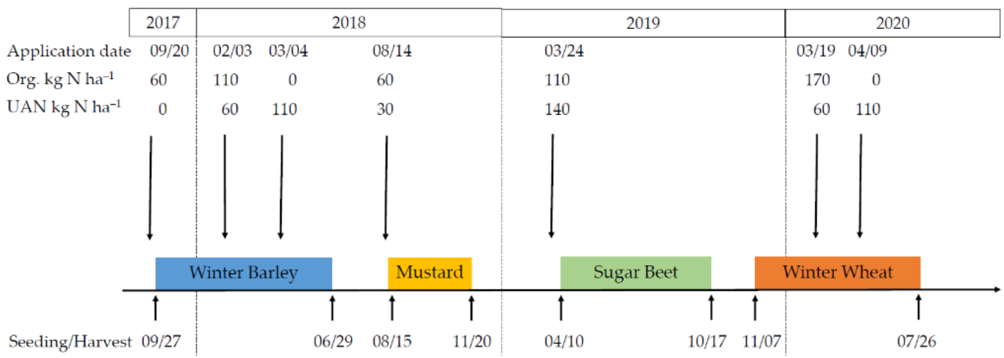

Figure 1. Dates of sowing, harvesting and N application in kg total N ha^{-1} (Org. = organic N apply in form of Cattle Slurry, Cattle Slurry–*Miscanthus* (five kg to one kg), Cattle Slurry–Wheat Straw (8.5 kg to 1 kg), Cattle Manure from *Miscanthus* shredded bedding, Cattle Manure from Wheat Straw shredded bedding; UAN = Urea Ammonium Nitrate) during crop rotation.

Table 2. Nutrient contents of the used treatments, respectively for each N apply.

	Test Parameter	Unit	CS [1]	CS–*Mis* [2]	CS–WS [2]	CM–*Mis* [2]	CM–WS [2]	UAN [1]	*Mis* [2]	WS [2]
Autumn 2017	Dry matter	%	9.7	20.9	16.4	32.7	25.4	-	87.8	86.2
	Organic matter	%	7.2	18.3	13.4	29.2	17.3	-	85.2	79.2
	Total N	$kg\ m^{-3}/kg\ t^{-1}$	4.6	4.2	5.1	5.6	8.3	-	1.7	6.3
	NH_4^+-N	$kg\ m^{-3}/kg\ t^{-1}$	2.3	1.4	1.1	0.5	0.2	-	<0.1	0.2
	NH_4^+-N in total N	%	50	33	22	9	2	-	5	3
	pH	value	7.3	8.4	8.2	8.3	8.2	-	6	6.8
	C/N	ratio	9	26	15	30	12	-	288	73
Spring 2018	Dry matter	%	9.2	20.1	16	32.5	26.1	-	87.8	86.2
	Organic matter	%	6.7	17.6	13	29.8	22.6	-	85.2	79.2
	Total N	$kg\ m^{-3}/kg\ t^{-1}$	4	3.9	4.7	6.5	6.4	358	1.7	6.3
	NH_4^+-N	$kg\ m^{-3}/kg\ t^{-1}$	1.8	1	1	0.7	1.3	90	<0.1	0.2
	NH_4^+-N in total N	%	45	26	21	11	20	25	5	3
	pH	value	7.7	8.1	7.8	8.2	8.3	-	6	6.3
	C/N	ratio	9.8	27	16	27	20	-	288	73
Autumn 2018	Dry matter	%	9.9	23.7	18.6	24.1	39.6	-	90.1	90.9
	Organic matter	%	7.3	21	15.6	21.8	22.1	-	86.9	86.3
	Total N	$kg\ m^{-3}/kg\ t^{-1}$	4.7	4.3	5.2	5.2	11.4	358	3	4.4
	NH_4^+-N	$kg\ m^{-3}/kg\ t^{-1}$	2.2	1.1	1.4	1.4	2.1	90	0.2	0.2
	NH_4^+-N in total N	%	47	26	27	27	18	25	7	5
	pH	value	-	-	-	-	-	-	-	-
	C/N	ratio	8.9	28.2	17.5	24.5	11.2	-	166	114.8
Spring 2019	Dry matter	%	7	20.9	15.5	22.8	17.5	-	90.1	90.9
	Organic matter	%	5.2	17.4	12.5	20.1	14.1	-	86.9	86.3
	Total N	$kg\ m^{-3}/kg\ t^{-1}$	3.8	2.9	3.8	4.6	5.2	358	3	4.4
	NH_4^+-N	$kg\ m^{-3}/kg\ t^{-1}$	2.1	0.4	0.4	0.8	1.6	90	0.2	0.2
	NH_4^+-N in total N	%	55	14	11	17	31	25	7	5
	pH	value	7.8	8.3	8.1	7.1	7.4	-	6.1	6.3
	C/N	ratio	7.9	35	19.2	25.3	15.7	-	166	114.8
Spring 2020	Dry matter	%	8.7	21.7	16.5	32.6	17.9	-	89.8	92.1
	Organic matter	%	6.7	18.3	13.3	28.4	12.6	-	86.8	87.8
	Total N	$kg\ m^{-3}/kg\ t^{-1}$	4.6	3.8	4.3	6.5	4.9	358	1.9	3.7
	NH_4^+-N	$kg\ m^{-3}/kg\ t^{-1}$	2.1	0.6	0.8	0.7	1.3	90	0.1	0.2
	NH_4^+-N in total N	%	46	16	19	11	27	25	5	5
	pH	value	7.3	8.4	8.3	8.3	8.1	-	6	6.4
	C/N	ratio	8.4	27.6	17.9	25.5	14.8	-	262.3	136.8

CS = Cattle Slurry, CS–*Mis* = Cattle Slurry–*Miscanthus* (five kg to one kg), CS–WS = Cattle Slurry–Wheat Straw (8.5 kg to one kg), CM–*Mis* = Cattle Manure from *Miscanthus* shredded bedding, CM–WS = Cattle Manure from Wheat Straw shredded bedding, UAN = Urea Ammonium Nitrate, *Mis* = *Miscanthus* shredding, WS = Wheat Straw shredding. Indication of the nutrient content in: [1] $kg\ m^{-3}$; [2] $kg\ t^{-1}$.

2.2. Soil Analyses

Per sampling and plot, six soil samples were taken for analysis. The analysis was carried out by forming a soil aliquot of each plot and determining inorganic N, soil microbial biomass C (MBC) and soil microbial biomass N (MBN). The N analysis quantified inorganic N as NH_4^+ plus NO_3^-, since NO_2^- was not detectable. For the preparation of analysis, soil samples were sieved at 2 mm and visible roots were removed. For the analysis of inorganic N, field-fresh soil was weighed into polyethylene bottles, filled with 100 cm^3 of 1% K_2SO_4 and then placed on an overhead shaker (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). All extracts were filtered (VWR 305; particle retention: 2–3 µm) and then stored until further analysis at −18 °C to avoid microbial transformation processes. Just before starting the analyses, extracts were defrosted rapidly to room temperature. Then, the content of inorganic N was determined with the AutoAnalyzer 3 from Bran + Luebbe GmbH Norderstedt, Germany.

For the analysis of MBC and MBN, chloroform fumigation-extraction [55,56] was used. For this, field-fresh soil was used for fumigation and for direct extraction. The fumigation was carried out in a vacuum desiccator (VWR International GmbH, Darmstadt, Germany) at 25 °C using ethanol-free chloroform ($CHCl_3$) for 24 h in the dark. The fumigated and non-fumigated samples were then extracted with 40 cm^3 of 0.5 M K_2SO_4 and placed on a horizontal shaker (IKA®–Werke GmbH & Co. KG, Staufen, Germany). All extracts were filtered (VWR International GmbH, Darmstadt, Germany; particle retention: 2–3 µm) and stored until analysis at −18 °C. Just before starting the analyses, extracts were defrosted rapidly to room temperature. In all extracts, organic C and total N were detected after combustion at 800 °C by using a Multi N/C 2100S (Analytic Jena, Jena, Germany). MBC was calculated as the ratio of extractable C (EC) and k_{EC}. EC is the difference between organic C extracted from fumigated soils and non-fumigated soils, whereas k_{EC} is a correction factor with the value of 0.45 [57] and represents the fraction of microbial C released in 24 h of fumigation. MBN gets calculated as the ratio of extractable N (EN) and k_{EN}. EN is the difference in organic N extracted from fumigated soils and non-fumigated soils, where k_{EN} is a correction factor with the value of 0.54 [56,58] and represents the fraction of microbial N. Inorganic N, MBC and MBN were extrapolated to one hectare by assuming a soil bulk density of 1.32 g cm^{-3}.

2.3. Plant Analyses

In each crop, during the vegetation period and at harvest time, plants were cut and used for determination of N uptake. Plant biomass were cut during vegetation period, five times when winter barley, one time when mustard, nine times when sugar beet and six times when winter wheat was cultivated. For winter barley and winter wheat, 1.2 m^2 and for sugar beet, 0.9 m^2 were taken for each sampling of each plot. At harvest date, 12 m^2 was sampled of each crop and of each plot by a plot combine (winter barley, winter wheat), by plot beet lifters (sugar beet) and by hand (mustard). Of the 1.2 and 0.9 m^2 plant samples, the whole plant biomass and of the 12 m^2 samples only a plant aliquot was placed in a drying oven at 50 °C until constant weight to calculate dry matter yield. Whole dried plant material was shredded and pulverised by using a cutting mill (Retsch, Haan, Germany) at 3000 rpm and by using a sieve of 0.25 mm. The C and N concentrations of each harvest-biomass were analysed by combustion and gas chromatographic analysis, using an elemental analyser (EA 3000 series, HEKAtech GmbH, Wegberg, Germany). Plant N uptake was calculated by using dry matter yield and N concentration of each sampling and then extrapolated to one hectare, without considering N uptake of the root biomass.

The amount of N released from applied fertilisers was estimated by subtracting the sum of plant N uptake of NoN treatments from the N uptake of the fertilised treatments at harvest. The total inorganic N value just before seeding of each crop and just after each harvest was included in the calculation by subtracting these differences from the N uptake of each treatment. This calculation does not take into account N losses in the form of ammonia and nitrous oxide and nitrate leaching.

The yields of each crop were calculated by extrapolating corn yield, sugar beet yield and total biomass of each 12 m^2 harvested plot to one hectare. The protein content of winter barley and winter wheat and the sugar content of sugar beet were analysed, after cleaning the grain and beet, using near-infrared spectroscopy.

2.4. Statistical Analyses

Statistical analyses and visualisations were performed using IBM SPSS Statistics 27 (IBM Ehningen, Ehningen, Germany). Normal distribution of data was tested using the Shapiro–Wilk test. Levene's test based on means was used to verify the homogeneity of variances. To identify treatment differences between three treatments, a one-way analysis of variance (ANOVA), following by a post hoc Tukey's HSD (honest significance difference) test were used. To identify differences between two treatments, a *t*-test was used. Tukey's HSD and *t*-test were performed separately for each experiment. When data were not normally distributed or no homogeneity of variance was detected, Welch test and Games–Howell test were used to identify differences for three or more treatments, Mann–Whitney–U test was used to identify differences between two treatments. A *p*-value of 0.05 was used as threshold for significant interactions.

3. Results

3.1. Plant N Uptake

The plant N uptake indicates differences in the N availability of the applied N fertilisers. The main crops (winter barley, sugar beet, winter wheat), fertilised with mineral N (UAN), showed the highest N uptake during the whole vegetation period of all main crops throughout the crop rotation, respectively, from the first sampling until harvesting (Figure 2). Only non-significant differences in N uptake from mineral or organic fertiliser were detected in the mustard catch crop. When crops were fertilised with Cattle Slurry (CS), lower amounts of N were plant available throughout the crop rotation as compared to mineral fertilisation (UAN). When cereals were cultivated (winter barley, winter wheat), tendency of greater amounts of N (non-significant) were taken up after CS fertilisation, compared to the other organic fertilisers (CS–*Mis*, CS–WS, CM–*Mis*, CM–WS).

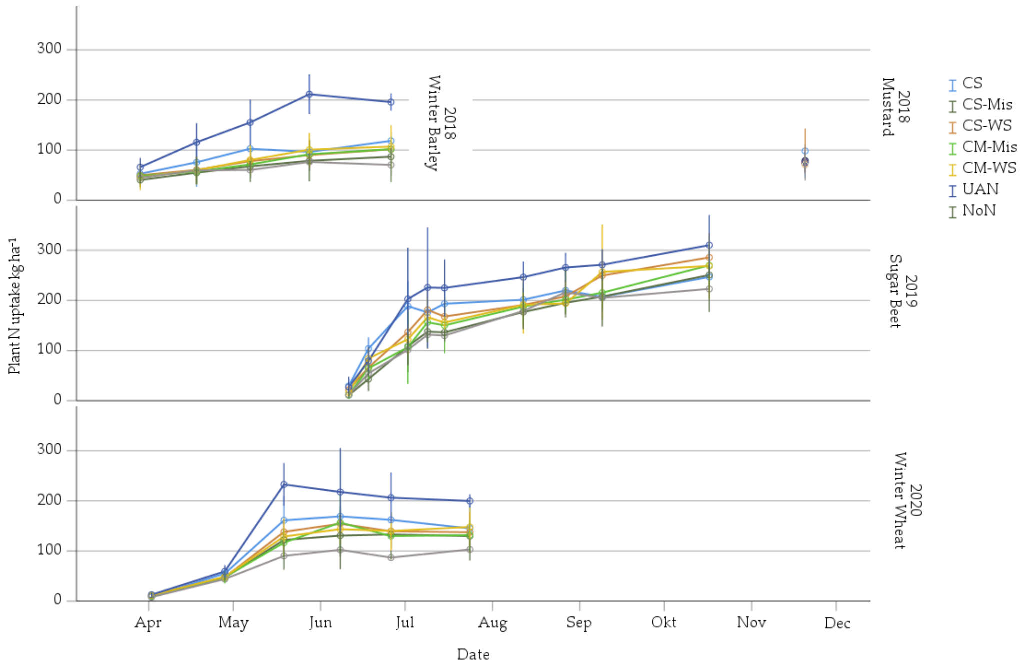

Figure 2. Plant N uptake during vegetation period (markings) from seeding to harvest of winter barley, mustard, sugar beet and winter wheat for each treatment (CS = Cattle Slurry, CS–*Mis* = Cattle Slurry–*Miscanthus* (five kg to one kg), CS–WS = Cattle Slurry–Wheat Straw (8.5 kg to 1 kg), CM–*Mis* = Cattle Manure from *Miscanthus* shredded bedding, CM–WS = Cattle Manure from Wheat Straw shredded bedding, UAN = Urea Ammonium Nitrate, NoN = no nitrogen applied). Error bars show standard deviations.

When *Mis* or WS were each mixed with CS and then applied as C-rich organic fertilisers to cereals and mustard, the N uptake at harvest was slightly lower (non-significant) compared to pure CS (Figure 2, Table 3). Thereby, the addition of *Mis* to CS induced a greater reduction of N uptake compared to the addition of WS to CS, respectively, in winter barley and winter wheat (Figure 2, Table 3). At the first samplings, the N uptake was stronger reduced than at the last samplings, respectively, for CS–*Mis* and CS–WS for both winter barley and winter wheat (Figure 2, Table 3).

In contrast, when both mixtures were applied to sugar beet, at harvest the N uptake was slightly greater (CS–*Mis* = 2%, CS–WS = 16%) compared to pure CS (Figure 2, Table 3). However, when CS–Mis was applied, in four of the first five samplings, it led to a statistically significant initial N immobilisation (*sampling one* = $F(2, 9) = 6.98$, $p = 0.015$; *sampling two* = $F(2, 9) = 25.39$, $p < 0.001$; *sampling three* = $F(2, 9) = 6.95$, $p = 0.015$; *sampling four* = $F(2, 9) = 3.06$, $p = 0.097$; *sampling five* = $F(2, 9) = 8.58$, $p = 0.008$), indicating a greater N immobilisation compared to CS–WS (only in one of the first five samplings) (Table 3).

Although the reductions of N uptakes, induced by added *Mis* and WS to CS, are mostly not significant, they are apparent, indicated by the means (Figure 2, Table 3).

When the two manure types were applied as fertiliser, no statistically significant differences between both manures in N uptake were detectable. In most samplings during vegetation period, N uptake was slightly lower after fertilisation of main crops with CM–Mis (Table 4).

Table 3. Percentage of N uptake of the two mixtures (CS–Mis = Cattle Slurry–*Miscanthus* (five kg to one kg); CS–WS = Cattle Slurry–Wheat Straw (8.5 kg to 1 kg)) to N uptake of Cattle Slurry (CS), to different samplings during vegetation period and at time of harvest. Listed for crop rotation, which consists of winter barley, mustard (catch crop), sugar beet and winter wheat, respectively. Different letters within a column and within each cultivar show significant differences. One-way ANOVA; $p < 0.05$; ns = not significant; $n = 4$.

		Sampling								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Harvest ***
Cultivar	**Treatment**	**N Uptake (% of CS)**								
Winter Barley	CS	100 ns	100 ns	100 ns	100 ns					100 ns
	CS–*Mis*	77 ns	73 ns	66 ns	82 ns					73 ns
	CS–WS	94 ns	80 ns	76 ns	93 ns					86 ns
Mustard	CS									100 a
	CS–*Mis*									88 b
	CS–WS									93 b
Sugar Beet	CS	100 a	100 a	100 a	100 ns	100 a	100 ns	100 ns	100 b	100 b
	CS–*Mis*	038 b	42 b	58 b	78 ns	70 b	88 ns	89 ns	100 b	102 b
	CS–WS	074 ab	64 b	73 ab	103 ns	87 ab	95 ns	95 ns	121 a	116 a
Winter Wheat	CS	100 ns	100 ns	100 ns	100 a	100 ns				100 ns
	CS–*Mis*	101 ns	85 ns	76 ns	77 b	82 ns				90 ns
	CS–WS	118 ns	87 ns	86 ns	91 ab	86 ns				95 ns
Cumulated	CS									100 ns
	CS–*Mis*									90 ns
	CS–WS									99 ns

* final sampling.

Table 4. Percentage of N uptake of the new type of Cattle Manure from *Miscanthus* shredded bedding (CM–*Mis*) to N uptake of conventional Cattle Manure from Wheat Straw bedding (CM-WS) to different samplings during the vegetation period and at time of harvest. Listed for crop rotation, which consists of winter barley, mustard (catch crop), sugar beet and winter wheat, respectively. Different letters within a column and within each cultivar show significant differences. One-way ANOVA; $p < 0.05$; ns = not significant; $n = 4$.

		Sampling								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Harvest ***
Cultivar	**Treatment**	**N Uptake (% of CM–WS)**								
Winter Barley	CM–*Mis*	106 ns	96 ns	89 ns	91 ns					95 ns
	CM–WS	100 ns	100 ns	100 ns	100 ns					100 ns
Mustard	CM–*Mis*									109 ns
	CM–WS									100 ns
Sugar Beet	CM–*Mis*	72 ns	77 ns	87 ns	94 ns	96 ns	98 ns	104 ns	84 ns	100 ns
	CM–WS	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns
Winter Wheat	CM–*Mis*	81 ns	99 ns	90 ns	109 ns	93 ns				89 ns
	CM–WS	100 ns	100 ns	100 ns	100 ns	100 ns				100 ns
Cumulated	CM–*Mis*									98 ns
	CM–WS									100 ns

* final sampling.

3.2. *Microbial Mineralisation–Immobilisation as Affected by Added Miscanthus Straw*

After mixing *Mis* or WS as a source of organic C with CS and applying it as fertiliser to cereals and mustard (catch crop), the fraction of mineralised N was in tendency slightly reduced, but not significant, compared to pure CS (*winter barley*: $F(2, 9) = 1.03$, $p = 0.396$; *mustard*: $F(2, 9) = 0.94$, $p = 0.448$; *winter wheat*: $F(2, 9) = 2.37$, $p = 0.149$). Therefore, for winter barley and winter wheat, the addition of *Mis* resulted in a slightly lower mineralised N fraction compared to WS addition, though the difference was not significant (*winter barley*: $T(6) = -0.88$, $p = 0.428$; *winter wheat*: $T(6) = -0.90$, $p = 0.405$) (Figure 3A).

After the application of CS–*Mis* and CS–WS as fertilisers to sugar beet, the fraction of mineralised N was in tendency slightly increased, though the difference was not significant, compared to pure CS (F (2, 9) = 0.90, $p = 0.442$). This increased N uptake of sugar beet after fertilisation with the mixtures (CS–*Mis*, CS–WS) may indicate an increased N release through mineralisation after initial N immobilisation by soil microorganisms.

When Cattle Manure (CM) from *Mis* as well as from WS were used as organic fertilisers, about the same fraction of both manures were mineralized for each crop. When they were applied to sugar beet, the fraction of N mineralised was greater than when they were applied to the cereals. When they were applied to the catch crop, the same amount of N was mineralised as became plant available from the soil N pool in the unfertilised ryegrass. Consequently, no additional N was mineralised compared to the plots without any N supplied (Figure 3B).

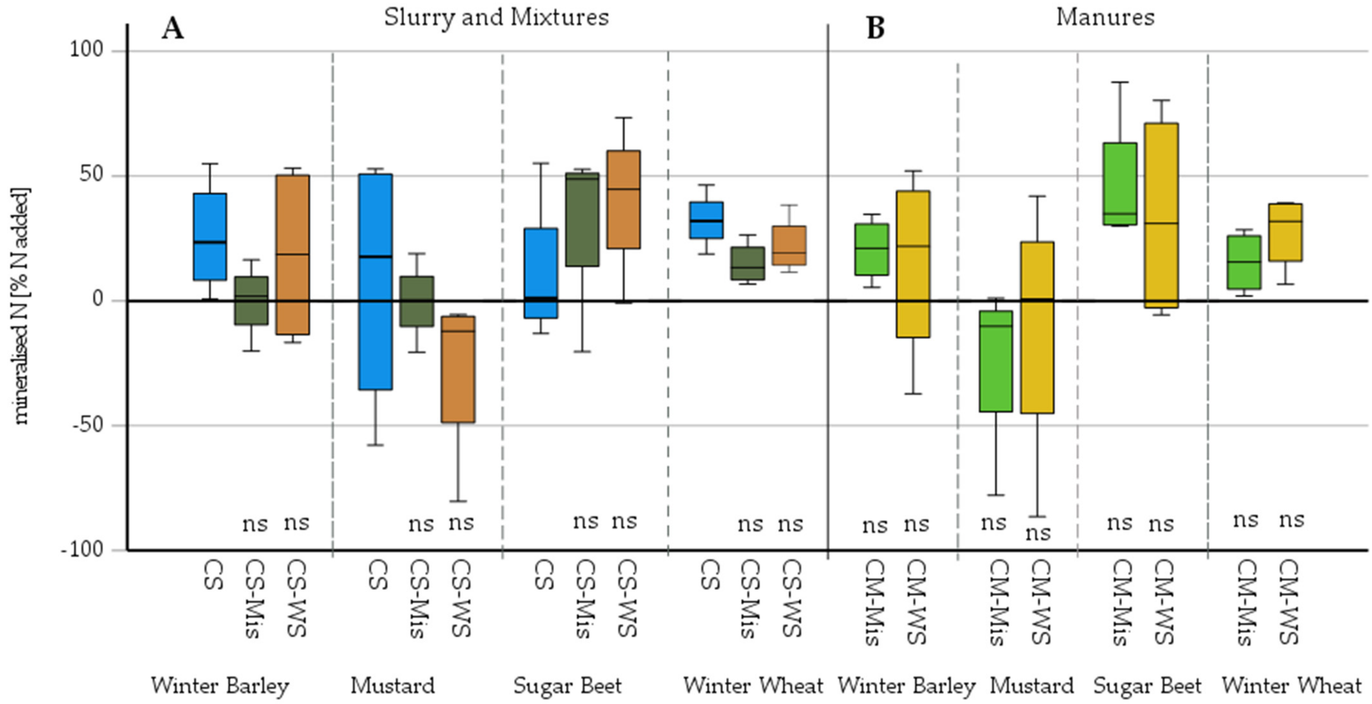

Figure 3. N mineralised expressed as % of N applied for winter barley, mustard (catch crop), sugar beet and winter wheat, calculated using the amount of N uptake and N apply of each cultivar. (**A**): CS = Cattle Slurry, CS–*Mis* = Cattle Slurry–*Miscanthus* (five kg to one kg), CS–WS = Cattle Slurry–Wheat Straw (8.5 kg to 1 kg); (**B**): CM–*Mis* = Cattle Manure from *Miscanthus* shredded bedding, CM–WS = Cattle Manure from Wheat Straw shredded bedding. The horizontal bars indicate the median and the whiskers indicate the 1.5 × IQR (interquartile range). No significant differences were indicated between the two types of mixtures (CS–*Mis*, CS–WS) and between the two types of manures (CM–*Mis*, CM–WS); $p < 0.05$, *t*-test; not significant; $n = 4$.

Cumulated for the total crop rotation, a slightly lower N fraction of the two mixtures was mineralised (CS–*Mis* = mean 12% ± SD = 12, CS–WS = 23% ± 12), although not statistically significant, from CS (CS = 29 ± 12) ($F(2, 9) = 1.49$, $p = 0.277$). Between both manure types, no statistically significant difference in the fraction of mineralised

N was detected (CM − *Mis* = 20% ± 7, CM–WS = 23% ± 12) ($T(6) = -0.27$, $p = 0.793$) (Figure 3A,B).

At the end of the field experiment, no significant differences in soil microbial biomass C (MBC) and N (MBN) were analysed. Neither between both mixtures (*MBC*: $T(6) = 0.18$, $p = 0.862$; *MBN*: $T(6) = 0.27$, $p = 0.800$), nor between both manures (*MBC*: $T(6) = 0.67$, $p = 0.529$; *MBN*: $T(6) = -0.43$, $p = 0.683$) (Figure 4A,B).

After the addition of C to CS, as *Mis* or WS and applied as organic fertilisers during crop rotation, the MBC and MBN were both slightly greater (Figure 4A,B), compared to CS only, although not statistically significant (*MBC*: $F(2, 9) = 0.47$, $p = 0.641$; *MBN*: $F(2, 9) = 1.15$, $p = 0.361$). Thus, the slightly lower N mineralisation of the mixtures compared to pure CS (Figures 2 and 3) is generally reflected by a slightly greater MB (Figure 4A,B). After the crop rotation and after the fertilisation with CS–*Mis*, MBC was 12% (156 kg ha^{-1}) and CS–WS was 9% (123 kg ha^{-1}) higher than the non-fertilised plots (Figure 4A), and MBN was 26% (60 kg ha^{-1}, CS–*Mis*) and 23% (54 kg ha^{-1}, CS-WS) higher than the non-fertilised plots (Figure 4B). Apparently, when *Mis* and WS were used for mixing with CS, soil microorganisms were able to assimilate slightly more N and C as compared to CS when they were used as fertilisers during a crop rotation.

When the manure types (CM–*Mis*, CM–WS) were applied as fertilisers, both the MBC and MBN were greater compared to the mixtures (CS–*Mis*, CS–WS), although not statistically significant (Figure 4A,B) (*MBC*: $F(3, 12) = 0.84$, $p = 0.499$; *MBN*: $F(3, 6.17) = 1.67$, $p = 0.270$)). Although there was no difference between the cumulated fractions of mineralised N after fertilisation with manures and mixtures (Figure 3 and description in text above), the MBC and MBN of manures were non-significantly, but slightly greater, compared to mixtures (Figure 4A,B). This indicates an increased uptake of applied manure N by the MB, compared to applied CS mixtures.

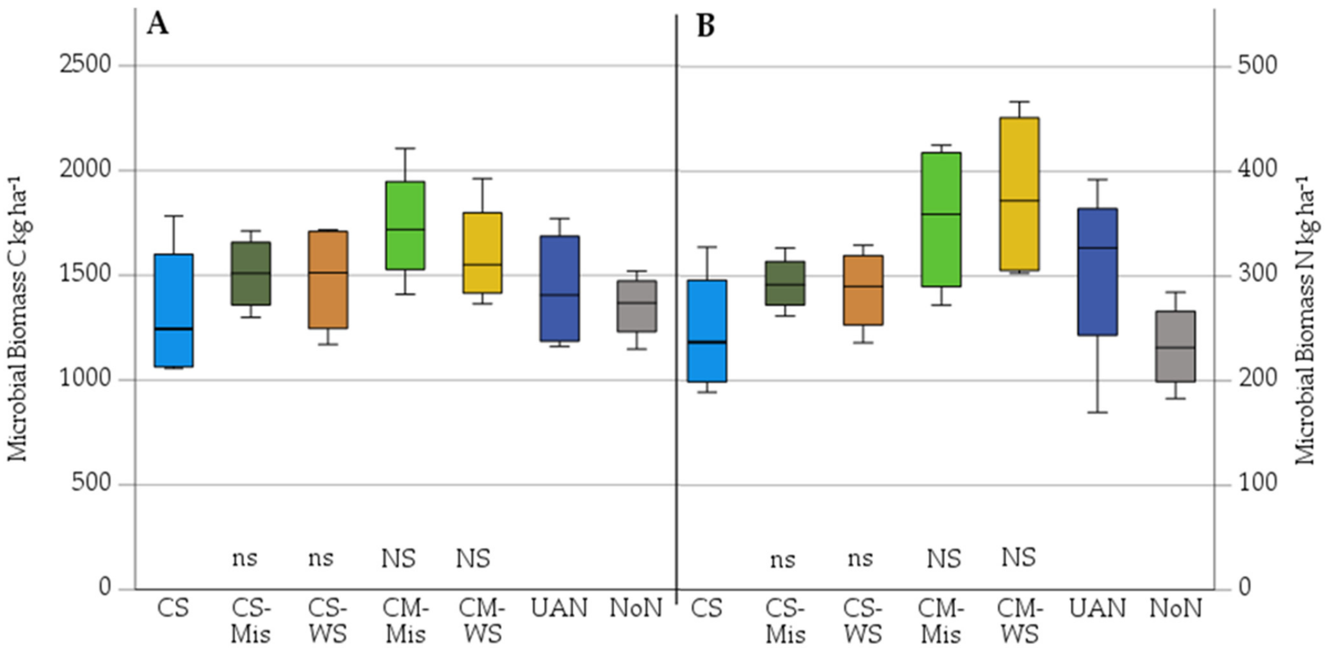

Figure 4. (**A**) Microbial biomass C kg ha^{-1} and (**B**) microbial biomass N kg ha^{-1} of the soils, following the application of different treatments (CS = Cattle Slurry, CS–*Mis* = Cattle Slurry–*Miscanthus* (five kg to one kg), CS–WS = Cattle Slurry–Wheat Straw (8.5 kg to 1 kg), CM–*Mis* = Cattle Manure from *Miscanthus* shredded bedding, CM–WS = Cattle Manure from Wheat Straw shredded bedding, UAN = Urea Ammonium Nitrate, NoN = no nitrogen applied). The horizontal bars indicate the median and the whiskers indicate the 1.5 × IQR (interquartile range). *t*-test, $p < 0.05$, ns = not significant; $n = 4$.

3.3. Inorganic N as Affected by Added Miscanthus Straw

The soil inorganic N indicates the amount of plant-available N during the crop rotation before, during and after the application of the different types of N fertilisers and in the plots without any N applied (Figure 5). After adding organic C in the form of *Mis* or WS to CS and then applied as fertilisers just before seeding of winter barley in September 2017, in the middle of December 2017, the inorganic N of the soils (soil layer 0–90 cm) was slightly reduced to an amount of 26 and 23 kg ha^{-1} (December 2017 in Figure 5) ($F(2, 4.213) = 4.92$, $p = 0.079$). Apparently, winter barley was not able to take up the entire amount of mineralised N from the CS, before the end of the vegetation period in 2017. The greater amount of inorganic N in autumn 2017, after CS fertilisation, was not detectable anymore at the end of winter (February 2018), so that N loss in the form of nitrate-leaching after CS fertilisation might have been at least 20 kg N ha^{-1} greater compared to all other organic fertilisers. Accordingly, the addition of C in the form of *Mis* and WS was effective in reducing N loss over the winter months (February 2018 in Figure 5).

In April 2018, when N demand of winter barley was high, N mineralisation of the mixtures was reduced, indicated by a reduced N uptake (Figure 2, Table 3) and by a reduced amount of inorganic N after fertilisation with CS–*Mis* and CS–WS, compared to pure CS (April 2018 in Figure 5) ($F(2, 5.27) = 4.10$, $p = 0.084$). After fertilisation with manures (CM–*Mis*, CM–WS) and after no fertilisation, the amounts of inorganic N were almost identical, compared to the CS mixtures. When both mixtures (CS–*Mis*, CS–WS) were applied just before seeding of the catch crop mustard, both caused a slightly lower amount of inorganic N compared to pure CS fertilisation ($F(2, 5.11) = 2.13$, $p = 0.212$), whereby the application of CS–*Mis* caused a slightly greater N immobilisation and thus lower amount of inorganic N, compared to CS–WS application ($T(6) = -1.70$, $p = 0.140$) (September 2018 in Figure 5). Although at the end of the vegetation period of 2018, mustard N uptake was slightly greater after CS fertilisation compared to the other treatments (Figure 2), the inorganic N was also slightly greater, compared to the amount of inorganic N of mixtures and of manures ($F(4, 7.11) = 0.43$, $p = 0.783$) (November 2018 in Figure 5). The amount of inorganic N after UAN fertilisation was even greater, but not significant ($F(5, 8.04) = 3.37$, $p = 0.062$) (November 2018 in Figure 5). This indicates again that the addition of *Mis* to CS was as effective as WS addition to CS in reducing the amount of potential N leaching, compared to pure CS. Neither the application of the mixtures nor the application of the manures to mustard resulted in a greater amount of inorganic N, compared to the mustard without any N applied (November 2018 in Figure 5).

In February of 2019, in the CS plots, the amount of inorganic N was 94 kg ha^{-1}, compared to 66 kg ha^{-1} in the CS–*Mis* and to 84 kg ha^{-1} in the CS–WS plots. This indicates an earlier start of N mineralisation of CS, compared to the mixtures, which were applied in 2018. Obviously, *Mis* and WS addition to CS also resulted in a lower N mineralisation in the year following application, whereas *Mis* addition caused a slightly slower N mineralisation compared to WS addition ($T(6) = -1.48$, $p = 0.190$).

In spring 2019 (sampling date May 27th), after the N fertilisers were applied to sugar beet, the added *Mis* and WS to CS caused N immobilisation. Thereby, the added *Mis* to CS caused a significant greater N immobilisation compared to added WS to CS, indicated by the amount of inorganic N of 69 kg ha^{-1} (CS–*Mis*), compared to the amount of inorganic N of 99 kg ha^{-1} (CSW–S) ($T(6) = -2.87$, $p = 0.028$) (May 2019 in Figure 5). Until the harvest of sugar beet, nearly all available N was taken up by, except after UAN application (October 2019 in Figure 5). After harvest of winter wheat, CS–*Mis* fertilisation caused a slightly lower amount of inorganic N compared to CS–WS fertilisation ($T(6) = -1.43$, $p = 0.201$) (August 2020 in Figure 5).

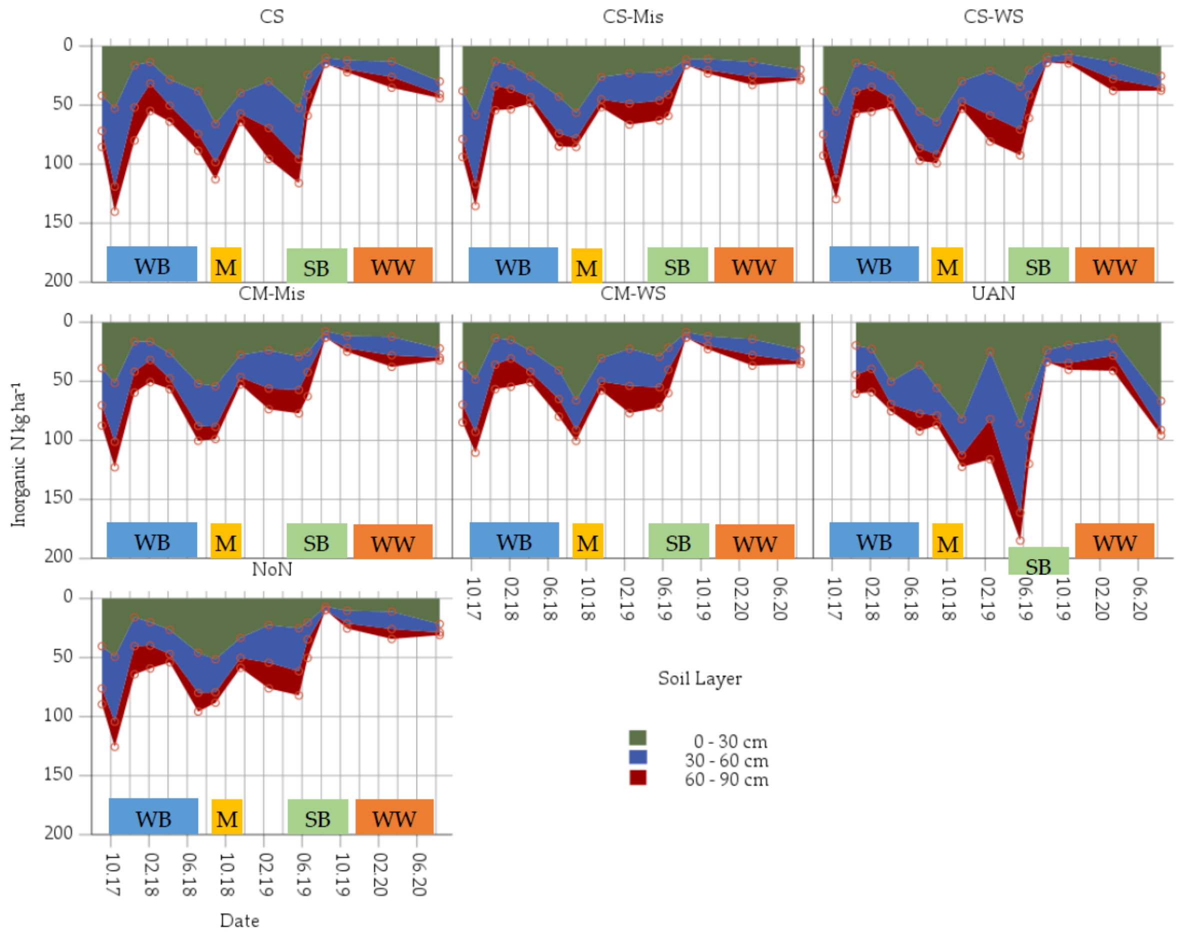

Figure 5. Inorganic N (NH_4^+, NO_3^-) in soil layers of 0–30 cm, 30–60 cm and 60–90 cm during crop rotation, consisting of winter barley (WB), mustard (M), sugar beet (SB) and winter wheat (WW) for each treatment (CS = Cattle Slurry, CS–*Mis* = Cattle Slurry–*Miscanthus* (five kg to one kg), CS–WS = Cattle Slurry–Wheat Straw (8.5 kg to 1 kg), CM–*Mis* = Cattle Manure from *Miscanthus* shredded bedding, CM–WS = Cattle Manure from Wheat Straw shredded bedding, UAN = Urea Ammonium Nitrate, NoN = no nitrogen applied). Labeling on the *x*-axis indicates the month and the year; data points represent the mean of $n = 4$.

3.4. Yield and Quality Parameters as Affected by Added Miscanthus Straw

The yield and quality parameters of the main crops were differently affected by the application of the types of N fertilisers (Table 5). When CS–*Mis* or CS–WS were applied as fertiliser to winter barley, both caused a slight reduction in protein content ($F(2, 9) = 2.02$, $p = 0.188$), compared to pure CS fertilisation. In contrast, the application of CS–*Mis* and CS–WS to sugar beet caused a significant greater beet yield of around 15 Mg ha^{-1}, compared to pure CS fertilisation ($F(2, 9) = 5.87$, $p = 0.023$). However, N availability to later vegetation stages after CS fertilisation must have been greater, indicated by a slightly greater amino-N content in the beets, compared to the amino-N content after fertilisation with mixtures ($F(2, 9) = 2.16$, $p = 0.17$). Thereby, the amino-N content after CS–*Mis* application was slightly, but non-significantly, lower compared to the CS–WS application ($T(6) = -2.38$, $p = 0.055$). When the mixtures were applied to winter wheat, both did not affect the grain yield nor the protein content, compared to pure CS fertilisation, $F(2, 9) = 1.82$, $p = 0.217$ (Table 5).

When both manure types were applied to winter barley, no differences were detected in grain yield ($T(6) = -0.17$, $p = 0.875$) and protein content ($T(6) = -1.85$, $p = 0.114$). Beet yields were not significantly different between the two manure treatments ($F(1, 6) = 5.68$, $p = 0.055$), yields were around 94 Mg ha^{-1}, with a corresponding sugar yield around 12 Mg ha^{-1}. Amino-N after CM–*Mis* application was slightly lower, compared to Amino-N after CM–WS application ($T(6) = -2.15$, $p = 0.075$). Apparently, the N fertilisers with a greater organic C content (Table 2) resulted in greater beet and sugar yields (Table 5). The N mineralisation in the plots without any N supply resulted in the same beet and sugar yield, compared to CS fertilisation (Table 5). In contrast, the beet yield was negatively affected by UAN application, with an amount of around 80 Mg ha^{-1}, apparently due to an excessively high N input (Table 5). This also led to an excessively high amino-N content of 37 Mmol kg^{-1}, making sugar extraction more difficult. Thereby, the sugar yield only reached an amount of about 10 Mg ha^{-1} (Table 5). When manure was applied to winter wheat, no difference in grain yield, but a slightly lower protein content after CM–*Mis* fertilisation was detected ($T(6) = -1.88$, $p = 0.109$), indicating a slightly lower N availability of CM–*Mis* compared to CM–WS (Table 5).

No statistical evidence for negative effects in yields and quality parameters are provided after application of each of the organic fertilisers compared to UAN fertilisation. Nevertheless, slight differences in the means of yields and quality parameters of winter barley and winter wheat are shown (Table 5).

Table 5. Yield and quality parameter of each of the cultivars of the crop rotation (winter barley, mustard, sugar beet, winter wheat) and for each treatment. The value represents the mean and standard deviation of $n = 4$. One-way ANOVA for CS, CS–*Mis* and CS–WS; *t*-test for CM–*Mis* and CM–WS, $p < 0.05$; different letters within a column show significant differences; ns = not significant (CS, CS-Mis, CS-WS); NS = not significant (CM-Mis, CM-WS).

Treatment	Winter Barley		Mustard	Sugar Beet			Winter Wheat	
	Corn Yield Mg ha^{-1}	Protein %	Biomass Yield Mg ha^{-1}	Beet Yield Mg ha^{-1}	Sugar Yield Mg ha^{-1}	AmN Mmol kg^{-1}	Corn Yield Mg ha^{-1}	Protein %
CS	5.0 ± 1.2 ns	11.0 ± 0.7 ns	2.3 ± 0.9 ns	84.3 ± 2.9 b	11.2 ± 4.0 b	21.2 ± 4.4 ns	8.9 ± 0.6 ns	10.0 ± 0.4 ns
CS–*Mis*	4.7 ± 1.1 ns	10.6 ± 0.1 ns	1.8 ± 0.3 ns	98.0 ± 4.4 a	12.1 ± 4.5 ab	14.3 ± 2.7 ns	8.4 ± 0.2 ns	9.4 ± 0.4 ns
CS–WS	4.9 ± 0.5 ns	10.4 ± 0.1 ns	1.6 ± 0.3 ns	99.3 ± 10.7 a	12.2 ± 4.7 b	16.9 ± 3.6 ns	8.9 ± 0.6 ns	9.5 ± 0.4 ns
CM–*Mis*	4.8 ± 0.7 NS	10.3 ± 0.3 NS	1.8 ± 0.6 NS	94.5 ± 12.2 NS	12.0 ± 4.5 NS	15.3 ± 3.0 NS	8.8 ± 0.5 NS	9.4 ± 0.4 NS
CM–WS	4.9 ± 1.0 NS	10.6 ± 0.1 NS	1.7 ± 0.3 NS	92.9 ± 9.4 NS	12.0 ± 4.4 NS	17.7 ± 3.8 NS	8.8 ± 0.7 NS	9.9 ± 0.3 NS
UAN	6.9 ± 1.7	14.1 ± 0.5	1.2 ± 0.3	79.7 ± 4.7	9.9 ± 3.8	37.3 ± 4.9	9.1 ± 0.2	12.0 ± 0.3
NoN	3.9 ± 0.5	10.2 ± 0.3	1.5 ± 0.2	85.7 ± 13.0	11.3 ± 4.4	16.6 ± 3.4	7.2 ± 0.4	9.1 ± 0.6

CS = Cattle Slurry, CS–*Mis* = Cattle Slurry–*Miscanthus* (five kg to one kg), CS–WS = Cattle Slurry–Wheat Straw (8.5 kg to 1 kg), CM–*Mis* = Cattle Manure from *Miscanthus* shredded bedding, CM–WS = Cattle Manure from Wheat Straw shredded bedding, UAN = Urea Ammonium Nitrate, NoN = no nitrogen applied.

4. Discussion

As an additional carbon (C) source in arable farming, we tested the use of *Mis* concerning N immobilisation–mineralisation, soil inorganic nitrogen (N), effects on yield and quality parameters of cultivated crops and microbial biomass build-up. We demonstrate that *Miscanthus* (*Mis*) as a C source was at least as effective as wheat straw (WS) in N immobilisation, with a tendency of a more pronounced impact on N immobilisation. This also resulted in a lower amount of soil inorganic N in the plots fertilised with the mixtures compared to the cattle slurry (CS) treatment, resulting in lower N losses over winter. We also demonstrate that the impact on N immobilisation–mineralisation was dependent on the duration of the N uptake of the respective crop.

4.1. *Miscanthus as C Source for Microbial N Immobilisation*

The fertilisers with *Mis* biomass (Cattle Slurry–*Miscanthus* mixture (CS–*Mis*) and Cattle Manure from *Mis* shredded bedding (CM–*Mis*)) were at least as effective as fertilisers with WS biomass (Cattle Slurry–Wheat Straw mixture (CS–WS) and Cattle Manure from WS bedding (CM–WS)) for N immobilisation, when they were applied to winter wheat and winter barley. This field trial showed a tendency of greater N immobilisation and lower inorganic N contents after application of *Mis* based fertilisers, whereas differences were not significant. In pot experiments, the effectiveness of *Mis* and WS biomass amended fertilisers for N immobilisation was already detected [34]. There, a tendency of a greater N immobilisation after application of fertilisers with *Mis* biomass (CS–*Mis*, CM–*Mis*) compared to WS amended fertilisers were explained by the microbial processes due to the biochemical differences in the carbon/nitrogen (C/N) ratio of the added substrates [33,59–61]. In our field experiment, the differences of N immobilisation between Mis amended and WS amended fertilisers were less obvious compared to those of Stotter et al. [34]. The *Mis* based fertilisers were characterised by a greater C/N ratio of between 25 to 35 for CS–*Mis* and CM–*Mis*, compared to a C/N ratio of 11 to 19 for CS–WS and CM–WS (Table 2). The differences in the C/N ratio between the *Mis* and WS based fertilisers are due to greater C/N ratio of the *Mis* raw biomass material of 166 to 288, compared to the C/N ratio of 73 to 137 of the WS biomass feedstock (Table 2). Additionally, the C amount of the CS–*Mis* mixture was enhanced by a lower mixing ratio of CS–*Mis* of 5:1, compared to CS–WS of 8.5:1, which could suggest a greater microbially available C from *Mis* biomass. The lower mixing ratio of CS–*Mis* also resulted in a lower content of total N of the CS–*Mis* fertiliser and therefore in greater application rates to the crops to achieve the same total application rate. Consequently, greater amounts of C due to CS–*Mis* compared to CS–WS and due to CM–*Mis* compared to CM–WS were applied to the soil. Nevertheless, no significant difference in N immobilisation was analysed.

Furthermore, contents of holocellulose and lignin of *Mis* and WS (*Mis*: holocellulose 70%, lignin = 14% to 19%; WS: holocellulose = 68 to 76%, lignin = 8 to 25%) and as well NH_4^+ content, a biochemical factor that strongly influences N availability [33,62–65], were almost identical for the *Mis* and WS based fertilisers (Table 2). Therefore, we suggest that available C input in the form of *Mis* may in principle appear to have greater effects on microbial N immobilisation than C input in form of WS. In this field trial, this potential effect was apparently overridden by other factors, such as the high soil organic matter (SOM) content of the previous grassland area.

When the organic fertilisers were applied to sugar beet, a greater amount of N uptake after fertilisation with mixtures and manures compared to pure CS was observed (Figures 2 and 3), which was not expected and apparently was attributed to a combination of influences. Missing precipitation for weeks with radiation intensity above the average from June to September of 2019, as well as amounts of precipitation below the average in the previous year, have led to drought and thus to extremely dry conditions also in the subsoil. Incorporated mixtures and manures consisted of a greater fraction of organic matter, compared to pure CS, which could have resulted in greater soil moisture, resulting in better N mineralisation, improved growth conditions and yield formation with a greater amount of N uptake in these plots in August and September of 2019 as organic matter increases water holding capacity in the soil. Furthermore, technical complications, which hindered the immediate incorporation of the applied CS, may have resulted in greater N losses from the CS in the form of ammonia than usual and thus resulted in a lower N uptake than expected. However, the C input of *Mis* in the form of mixtures and manures shows that *Mis* is at least as effective as WS in providing inorganic N over the winter months.

Obviously, the period of one crop rotation was too short for analysing clear differences between both C-rich amendments. The extension of the experiment over a longer period of time with further C input could increase SOM and potentially result in measurable differences between *Mis* and WS addition.

4.2. Miscanthus as C Source for Microbial-Derived C Sequestration

The greater microbially available C input in form of *Mis* compared to WS for mixtures and manures, respectively, was not reflected in a greater microbial biomass (MB), both for microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) (Figure 4A,B). In contrast, the greater C input of *Mis*–manure (CM–*Mis*) compared to CS–*Mis* mixture and the greater C input of WS–manure (CM–WS) compared to CS–WS-mixture each resulted in a slightly greater MBC, though not significant. This indicates that the amount of C input could not have been the only factor influencing the slightly greater MBC in the plots fertilised with manures compared to the MBC of the plots fertilised with mixtures, but rather the characteristics of the manures were responsible for stronger growth of MB compared to the mixtures (Figure 4B). Other potential factors influencing the MB such as the pH value of the mixtures and manures (pH-values: CS-*Mis* = 8.3, CS-WS = 8.1, CM-*Mis* = 8.0, CM-WS = 8.0 (Table 2)) and natural site factors (soil type, soil temperature, soil moisture) [66] can be excluded as potential factors influencing MB, based on the fact that they were comparable of each fertiliser and field plot. Possibly, the period during which the manure accumulated in the animal barn could have led to a stimulation of the manure-microorganisms, which had a positive effect on the soil MB.

Nevertheless, both the mixture and manure from *Mis* each promote, just like the mixture and manure from WS, the soil MB, though not significantly. The C and N supply by the *Mis* based fertilisers caused a promotion of soil MB, which contributes to the formation of microbial necromass [67]. Microbial necromass has an essential role in the formation and stability of SOM and is thus a key component of C sequestration [68–71]. Therefore, the soil fertility can be improved, the SOM can contribute to a reduction of N losses and the promotion of MB contributes to ecological soil functioning for the resilience of arable soils [72]. Especially, regions with high organic N occurrences, for example, already formed by excretions in animal farming, have the potential for humus build-up, because N is an essential N compound for the build-up of SOC. However, humus build-up cannot be exploited without C availability, thus *Mis* biomass can provide an additionally essential C source for many agricultural regions. Farmers can cultivate it on semi-productive arable land to develop a C source regionally [35] and can be cultivated as a subsidised crop [37]. The utilisation as bedding materials for animals is more economical due to the cascading than to mix the Cattle Slurry with *Mis* by an additional working step.

The cultivation of *Mis* in areas with high livestock farming and thus with a high demand of bedding or biomass for bioenergy production can buffer the demand for cereal C in arable regions [73] and thus counteract the continuous SOM losses [13]. In arable regions, *Mis* cultivation on low-yield potential sites and thus C production counteracts the dependence on external C sources such as imported organic fertilisers like slurry or manures of predominantly farming areas. Furthermore, *Mis* can contribute to fulfilling the expected increase in demand for bedding materials [74], as a result of the increasing transformation of animal farming from slatted floors to bedding with straw.

In soils with high available N contents, *Mis* could find suitability for N immobilisation as high C amendment without any addition of excreta. Wheat Straw and spruce sawdust were already designated and applied as high C amendments to prevent N losses [32,33]. Especially in soils with a high potential of N mineralisation, C application could be a functional step in arable farming to contribute to reducing N losses.

However, the induced N immobilisation after the addition of C-rich plant material is not new knowledge at all [62,75], but can be increasingly implemented in the future as a tool to counteract SOM degradation as well as nitrate loss on susceptible arable land. The special cultivation of C-rich plants in the form of perennial crops such as *Mis* can be used as a component of future arable farming systems to ensure the yield capacity of soils. In addition to *Mis*, other greening measures and non-used fields of grassland can be transferred into a usage that aims at C production, which either provides bedding material or is directly applied to the cropland for SOM build-up. Therefore, unused grasslands are suitable, as they usually have to be cut once per year.

5. Conclusions

Integration of *Miscanthus x giganteus* L. (*Mis*) in arable farming for provision of ecosystem services and utilisation as an additional carbon (C) source of *Mis*-amended fertilisers results in different nitrogen (N) dynamics, depending on the crop. The application of fertilisers amended with *Mis* was at least as effective as fertilisers amended with Wheat Straw (WS) biomass in the immobilisation of inorganic N from Cattle Slurry (CS) and Cattle Manure (CM), when they were applied to cereals. Furthermore, both *Mis*-amended fertilisers (Cattle Slurry–*Miscanthus* mixture (CS–*Mis*) and Cattle Manure from *Mis* shredded bedding (CM–*Mis*)) led to better growth conditions, compared to CS application, under the given dry weather conditions during parts of the crop rotation, indicated by the N uptake and yield of sugar beet. Application of *Mis* fertilisers during a three-year crop rotation caused a temporary significant reduction of inorganic soil N and thus reduced N loss compared to CS. Compared to WS addition to CS, added *Mis* to CS caused an identical reduction of inorganic N during winter. We suggest that application of C-rich fertilisers over a longer period of time may have greater influences on soil organic matter (SOM) and on the reduction of inorganic N over winter months than has been analysed during this rotation period.

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