The fate of nitrogen derived from mown wetland biomass in a swampy river valley landscape

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SUMMARY

Wetlands provide a natural environment for nutrient attenuation; however, these ecosystems may also be used as a source of nutrients for soil fertilisation. Anaerobic digestion (AD) of mown plants from wet areas is a promising option to solve the problem of harvested biomass, while the digestate produced during the AD process can be a valuable nitrogen (N) fertiliser. An incubation experiment was run to investigate the effect of fertilising with digestates produced from four wetland plant species (reed sweet-grass, common reed, tufted sedge, reed canary grass) on inorganic-N dynamics in arable soil typical for the region. The amount of N in all digestates was similar and ranged from 46.8 ± 5.6 to 61.5 ± 3.1 g kg^{-1} (dw). The inorganic-N concentration in the soil increased during the first two weeks, mainly due to a reduction in NH_4-N. Rapid NO_3-N production led to the amount of NO_3-N almost doubling as a result of fertilisation. In all amended soils the N dynamics were similar and did not differ from those in soil fertilised with digestate derived from maize. The incorporation of N from biomass harvested in wetlands into soils on the adjacent arable land could play an important role in the N cycle of a swampy river valley landscape by reducing the need for additional N inputs and thus reducing the transfer of N from agricultural uplands to the river.

KEY WORDS: arable land, digestate, fertiliser

INTRODUCTION

Wetlands are recognised as essential ecosystems providing many goods and services at global and local scale such as climate regulation (Mitsch *et al.* 2013), climate change mitigation (Fossey & Rousseau 2016), carbon storage (Fennessy *et al.* 2018), biodiversity maintenance (Gibbs 2000), flood attenuation (Watson *et al.* 2016), recreational and cultural services (Schläpfer *et al.* 2015), and water quality improvement (Saunders & Kalff 2001). Wetlands are also regarded as a source of food and fodder within the broader context of the paludiculture concept (Wichtmann & Joosten 2007). On the other hand, many natural wetlands located in agricultural landscapes are threatened by the intensification of agriculture, which leads to diffuse transfer of N from cropped fields to these vulnerable ecosystems. Excess nutrients contribute significantly to the eutrophication of wetlands, which results in negative changes in the species composition and structure of their vegetation (Keddy 2000). Negative effects on plant composition through expansion of aggressively invasive species such as *Phragmites australis* and *Phalaris arundinacea* (Kotowski & Piórkowski 2005) are also caused by the withdrawal of agricultural management from wetlands where biomass harvesting for animal bedding, combined with grazing, formerly played an important role in maintaining the biodiversity of plant species and nesting birds by maintaining a vast open area. Nowadays, the most frequently applied management measure to maintain biodiversity and preserve the remaining natural wetlands is mowing (Kołos & Banaszuk 2013).

Recent research has identified the two additional ecosystem services of energy and fertiliser supply via the utilisation of biomass harvested from wetlands for biogas generation. The addition of grass silage collected for landscape management in nature conservation areas (Blokhina *et al.* 2011) and buffer strips (Gołkowska *et al.* 2016) to animal slurry undergoing anaerobic digestion results in volumetrically higher methane (CH_4) and biogas production, although with lower CH_4 concentration (Moset *et al.* 2017). The biogas and specific methane yields of reed canary grass and common reed are much lower than those obtained from maize silage, but comparable to the results of AD processing of pig manure, indicating that wetland plants can be considered as a valuable and inexpensive co-substrate (Roj-Rojewski *et al.* 2018a). It has been proved that digestate from the AD process is a high-quality fertiliser (Alburquerque *et al.* 2012a, Sigurnjak *et al.* 2017). The use of digestate obtained

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by AD processing of biomass harvested from wetlands as a substitute for mineral fertilisers allows wetlands to function not only as N sinks that attenuate diffuse agricultural losses (Tanner & Kadlec 2013) but also as sources of nutrients for agriculture.

In the AD process, total nitrogen (TN) is conserved but organic-N compounds are mineralised and this leads to an increase of inorganic-N forms, mainly NH_4-N (Möller & Müller 2012). Up to 13 % of N is lost in gaseous forms during application of digestate to the land and up to 15 % of the applied N is lost by leaching (Nicholson *et al.* 2017). The remaining N may be fixed in 2:1 type vermiculite, montmorillonite clay minerals and organic matter (due to the positive charge of NH_4^+); transformed into microbial N; or converted under aerobic conditions to nitrates (NO_3^-) within the process of nitrification. Plants take up N mainly as NO_3-N, whereas inorganic N in digestate occurs primarily as NH_4-N. Therefore, in order to understand the fate of N in the landscape, it is essential to know about rates of N transformation and recovery. The nitrification rate is a key determinant of N losses. High nitrification rates may result in high N losses via leaching, while reduced nitrification may enhance ammonia (NH_3) volatilisation. The composition and stability of digestate vary greatly depending on the substrates used for co-digestion, the retention time and conditions within the digester; and digestate stability and C/N quotient influence the pattern and rate of N transformation. In soils fertilised with unstable digestates, the rate of N immobilisation is higher than in soils amended with stable by-products of the AD process. NH_4-N applied to the soil with digestates oxidises rapidly during the first two weeks after soil amendment and, depending on the digestate, 41–84 % of the TN added is converted through nitrification (Alburquerque *et al*. 2012b, de la Fuente *et al*. 2013). Soil type and additions of other soil amendments, as well as the time and rate of application, are also main drivers of nitrification in soil fertilised with digestate (Loria & Sawyer 2005, Rigby & Smith 2013, Martin *et al*. 2015). The complex interactions between soil mineral fractions, organic matter (OM) and microbial activity affect nitrification rates, and the process may thus be more closely related to soil OM content than to its texture (Rigby & Smith 2013). However, the results obtained by Cavalli *et al.* (2017) suggest that clay composition plays the most important role in NH_4-N immobilisation.

Most studies on the transformation of N added with digestate to the soil have been performed on digested animal slurries with added plant or animal waste and digestates obtained from energy crops (Loria & Sawyer 2005, Grigatti *et al*. 2011, Alburquerque *et al.* 2012b, de la Fuente *et al*. 2013, Rigby & Smith 2013, Martin *et al*. 2015). In these studies the digestates, especially their liquid fractions, have provided higher amounts of plant-available N when compared to raw slurries and solid manures (Grigatti *et al*. 2011, Johansen *et al*. 2013) and similar amounts when compared to composted animal wastes (Gómez-Brandón *et al*. 2016). However, in the case of digested and raw pig manure, this difference was not found (Loria & Sawyer 2005).

Although high nitrification rate in soils fertilised with digestate may be a positive feature of these biowastes (Johansen *et al*. 2013), there is growing concern about N losses due to the rapid increase of NO_3-N concentration in the soil. Whereas most studies have focused on greenhouse gas emissions after fertilisation (Johansen *et al*. 2013, Martin *et al*. 2015, Nicholson *et al*. 2017), a few have investigated other effects such as leaching. For example, Goberna *et al*. (2011) and Nicholson *et al*. (2017) have shown that the rapid increase of NO_3-N concentration in soil treated with digestate results in high rates of N leaching, comparable to those occurring in soils fertilised with pig slurry, and much higher than the losses from soils amended with pig manure or compost.

There is a gap in knowledge about N transformations in soil amended with digestates produced from grasses and other plants derived from landscape management operations, and little is known about the environmental effects of such digestate applications. Therefore, the present study aimed to investigate the effects of digestates derived from wetland plants (reed sweet-grass, common reed, tufted sedge and reed canary grass) on N dynamics in amended soils under controlled temperature and moisture conditions, in comparison with the widely-used digestate derived by AD processing of maize silage.

METHODS

Soil and digestates characteristics

Soil for the experiment (loamy sand consisting of 83 % sand, 16 % silt and 1 % clay) was collected from an arable field near the city of Białystok (53° 17' N, 23° 11' E, 147 m a.s.l.) from a depth of 0–0.2 m. The soil sample used for the incubation experiment was air-dried, homogenised and sieved through a 2 mm mesh. All plant remains and roots were removed.

The digestates were obtained from the laboratory experiment on the bio-methane potential of four species: reed sweet-grass (*Glyceria maxima* (Hartm.) Holmb.; RSG), common reed (*Phragmites australis*

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(Cav.) Trin. ex Steud.; CR), tufted sedge (*Carex elata* All.; TSD) and reed canary grass (*Phalaris arundinacea* L.; RCG). Plants were harvested from wetlands in the Narew River Valley, Poland, the natural anastomosing river system protected as the Narew National Park. Plants were harvested in the late summer of 2015. The plant material was dried for 24 h at room temperature and silaged without any additives, as required for the bio-methane potential test and to simulate the treatment under the conditions for biomass harvest and utilisation imposed by protection measures for the National Park. The digestate used for comparison was obtained from a commercial biogas plant fed with maize silage (MS), chicken droppings (10 %) and potato pulp (10 %).

Soil analyses

Soil moisture content was determined by drying 10 g of soil to constant weight at 105 ± 2 °C. The NO_3^--N and NH_4^+-N contents were determined by UV-1800 spectrophotometer (Shimadzu, Japan) after extraction of a 10 g soil sample with 100 mL of 1 % K_2SO_4 for 24h (PN-R-04028:1997). Particle-size distribution was determined according to the hydrometer method (PN-R-04032:1998). A HQ40D meter (Hach, USA) was used to measure electrical conductivity (EC) in water extract with soil:distilled water ratio 1:2.5 (w/v as g/ml) and pH in 0.1M KCl (soil:potassium chloride ratio 1:2.5; w/v as g/ml). Bulk density was determined for undisturbed soil samples in a 100 cm^3 steel cylinder. Organic carbon was measured in a TOC-L analyser (Shimadzu, Japan) and total Kjeldahl nitrogen (TKN) was determined by the Kjeldahl method in a Vapodest 50s analyser (Gerhardt, Germany). After nitric acid/hydrogen peroxide microwave digestion in ETHOS One (Milestone s.r.l., Italy), the content of P was determined by the ammonium metavanadate method (Ostrowska *et al*. 1991) using a UV-1800 spectrophotometer (Shimadzu, Japan) and K content was measured using a flame photometer (BWB Technology, USA). All measurements were performed in triplicate. Properties of the soil before incubation are presented in Table 1.

Digestate analyses

Total solids (TS) and volatile solids (VS) were measured according to standard methods (APHA 1999). Total organic carbon (TOC) was measured in a TOC-L analyser (Shimadzu, Japan) and total Kjeldahl nitrogen (TKN) was analysed in fresh samples by the Kjeldahl method in a Vapodest 50s analyser (Gerhardt, Germany). The analyses were run in triplicate. Chemical and physicochemical characteristics of digestates used for incubation are shown in Table 1. The results are given on a dry weight basis.

Incubation experiment

Just before the experiment distilled water was added to air-dried, sieved (2 mm) and gently homogenised soil to maintain moisture at 60 % of water-holding capacity (WHC). Five fresh digestates were added to the soil in amounts equivalent to a field application of 170 kg N ha^{-1}, which is the recommended annual

Table 1. Main physical and chemical characteristics of soil used for the incubation experiment and characteristics of the digestate from anaerobic digestion of reed sweet-grass (RSG), common reed (CR), tufted sedge (TSD), reed canary grass (RCG) and maize (MS); mean value ± standard deviation, data expressed on a dry weight basis. TS = total solids, VS = volatile solids, TKN = total Kjeldahl nitrogen, TOC = total organic carbon, TP = total phosphorus, TK = total potassium, ρ = bulk density, n.a. = not analysed

Property	Soil	Digestate from anaerobic digestion of:				
		RSG	CR	TSD	RCG	MS
TS (%)	n.a	4.94±0.19	6.28±0.11	5.63±0.02	5.37±0.03	7.21±0.13
VS (%TS)	n.a	72.99±0.19	71.10±0.11	75.27±0.02	75.67±0.03	72.59±0.28
TKN (g kg^{-1})	0.90±0.02	53.8±3.7	46.8±5.6	48.9±1.5	53.7±1.0	61.5±3.1
TOC (g kg^{-1})	15.46±1.08	379.4±15.4	353.0±8.3	393.6±7.7	389.0±8.8	389.5±12.1
TP (g kg^{-1})	0.86±0.06	12.8±1.0	9.4±0.4	9.3±0.3	10.9±0.4	11.8±0.3
TK (g kg^{-1})	1.01±0.04	74.6±8.9	61.4±0.8	72.8±6.0	61.9±0.5	50.0±1.2
pH	4.75±0.12	n.a.	n.a.	n.a.	n.a.	n.a.
ρ (g cm^{-3})	1.51±0.05	n.a.	n.a.	n.a.	n.a.	n.a.

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rate of N application for organic fertilisers (Journal of Laws 2007). The exact rates of digestate added to 77 g of soil were calculated on the basis of measured bulk density equal to 1.51 g cm^{-3} assuming a cultivation depth of 0.2 m. The unamended soil was used as a control. The mixtures of soil and digestates and controls were placed in 100 ml plastic vessels without drainage holes and closed with breathable Parafilm to avoid anaerobic conditions through ensuring gas exchange and stable moisture at the same time (de la Fuente *et at*. 2010). Despite the use of Parafilm, the moisture content of the soil was checked every 3–4 days by weighing and 60 % WHC was restored if needed by adding distilled water. Aerobic incubation was run in darkness at 25 ± 1 ºC for 56 days. This temperature is considered optimal for N mineralisation-nitrification transformation processes (Smith *et al*. 1998). The samples of fresh soil-digestate mixtures and control in triplicate were taken for inorganic N (NO_3-N, NH_4-N) analyses just after soil amendment (Day 0) and then periodically on Days 2, 7, 14, 28, 42 and 56. Additionally, samples for TKN and TOC were taken on Days 0, 2 and 56.

The calculation of net N-mineralisation, N mineralisation from digestate and nitrification conversion was taken from Alburquerque *et al*. (2012b). Net N-mineralisation in digestate-fertilised and control soils were calculated according to the following equation:

$$N_{min} = inorgN_{d56} - inorgN_{d0} \quad [1]$$

where N_{min} was the dry soil net N mineralisation expressed in mg kg^{-1}, $inorgN_{d56}$ was the amount of inorganic N in the soil after 56 days of incubation and $inorgN_{d0}$ was the amount of inorganic N in the soil after digestate application at Day 0. The N mineralisation from digestate was calculated as:

$$NN_m = 100 \cdot \frac{[(iN_{d56} - iN_{d0})_{soil+dig} - (iN_{d56} - iN_{d0})_{soil}]}{TN_{added}} \quad [2]$$

where N_m was the N mineralisation from digestate in %, iN_{d56} was the amount of inorganic N in the amended (*soil+dig*) or unamended soil after 56 days of incubation, iN_{d0} was the amount after digestate application at Day 0, and TN_{added} was the amount of N added with the digestate. The nitrification rate was calculated according to the following formula:

$$N_c = 100 \cdot \frac{[(NO_3{-}N_{d56} - NO_3{-}N_{d0})_{soil+dig} - (NO_3{-}N_{d56} - NO_3{-}N_{d0})_{soil}]}{TN_{added}} \quad [3]$$

where N_c was the nitrification rate of N from digestate in %, NO_3-N_{d56} was the amount of NO_3-N in the soil after 56 days of incubation, NO_3-N_{d0} was the amount of NO_3-N in the soil after digestate application at Day 0, and TN_{added} was the amount of N added with digestate.

Data analysis

The significant differences in chemical properties amongst soils amended with different digestates were assessed with two-way analysis of variance (ANOVA) using type of digestate and incubation time as fixed factors. A multifactorial linear model (fixed effects linear model) was used to measure the main effect of each factor and the interactions between factors. Differences between means were determined using Tukey's test. In the case of NO_3-N and time as factors as well as in the case of pH, time and digestate treated as single factors, a non-parametric comparison using the Kruskal-Wallis test was performed instead of ANOVA because of variance inhomogeneity. The level of accepted statistical significance was $p < 0.05$. The homogeneity of variance and normality was checked prior to ANOVA using the Levene and Shapiro-Wilk tests, respectively. When data failed the test, they were adjusted by log-transformation. All the statistical analyses of data were performed using STATISTICA 12 software (StatSoft).

RESULTS

Nitrogen transformation in soil

All of the digestates we studied supplied NH_4-N to the soil, resulting in significantly higher NH_4-N content in all soils with amendments than in soils without digestate at Day 0 (ANOVA $F_{5,84} = 28.988$, $p = 0.00$). In the control soil the initial amount of NH_4-N was 14.8 ± 0.6 mg kg^{-1} while in soils treated with digestates it ranged from 55.77 ± 6.23 mg kg^{-1} (soil ± RSG) to 70.55 ± 4.96 mg kg^{-1} (soil ± RCG) in the order: RCG > CR > MS > TSD > RSG; however, there were no significant differences among the soils fertilised with the studied digestates (ANOVA $F_{5,84} = 28.988$, $p = 0.00$). The NH_4-N concentration in soils amended with digestates was significantly higher ($p < 0.05$) than in the control throughout incubation until Day 42, when the NH_4-N content in both untreated and treated soils fell to zero and stayed at this level until the end of incubation at Day 56 (Figure 1). The overall pattern of NH_4-N dynamics was similar in all soils, with a rapid decrease to a range of 9.22 ± 4.12 mg kg^{-1} (soil + RCG) to 19.02 ± 0.66 mg kg^{-1} (soil + MS) at Day 14 followed by slower reduction until Day 42.

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In contrast to NH_4-N, the NO_3-N content was initially very low and similar in all treated soils and the control soil. In untreated soil, the NO_3-N concentration rose from 5.23 ± 0.30 mg kg^{-1} at Day 0 to 18.60 ± 1.14 mg kg^{-1} during the first week and was stable throughout next 7 days, then increased to 42.65 ± 3.01 mg kg^{-1} at Day 28. In soils with digestates, the initial NO_3-N levels were also about 5 mg kg^{-1} and increased fourfold after one week. As opposed to untreated soil, the NO_3-N concentration in samples treated with digestates rose rapidly from Day 7 to Day 14 and further to Day 28. At the end of incubation, the NO_3-N content in soils fertilised with digestates was significantly higher ($p < 0.05$) than the NO_3-N concentration in soils without amendment (Figure 1).

The inorganic-N (NH_4-N and NO_3-N) dynamics in the soils mainly followed a similar pattern in both treated and untreated soils; however, some differences occurred during the first two weeks of incubation. In the control, inorganic-N increased during the first week of incubation and reached 23.75 ± 1.15 mg kg^{-1}, then returned to the initial value after the following week of the experiment. A rapid increase of inorganic-N content (to 50.26 ± 1.59 mg kg^{-1}), concomitant with a high rate of nitrification, was observed in the control at Day 28; in the next two weeks the inorganic-N concentration decreased to 36.06 ± 8.01 mg kg^{-1} then remained stable until the end of incubation. A similar pattern of inorganic-N changes was observed for soils fertilised with RSG and TSD, while in the case of soils treated with RCG, CR and MS a slow decrease was observed during the first two weeks of incubation (Table 2). A very rapid increase of inorganic-N occurred at Day 28 in all of the soils studied. In the subsequent weeks of incubation, inorganic-N content slowly decreased in soils amended with RSG, RCG and TSD and increased slightly in soils treated with CR and MS. Although there were some differences in the patterns of inorganic-N dynamics, the concentrations were not significantly different among the soils treated with digestates at most sampling dates, but inorganic-N content was significantly lower in the control. The amount of inorganic-N in soil fertilised with RSG was significantly ($p < 0.05$) higher than in the control and soils treated with other digestates at Day 7 only. Net N-mineralisation (N_{min}) after 56 days of incubation was +13.53, +2.10, +1.42, -8.07 and -19.53 mg kg^{-1} for soil fertilised with RSG, CR,

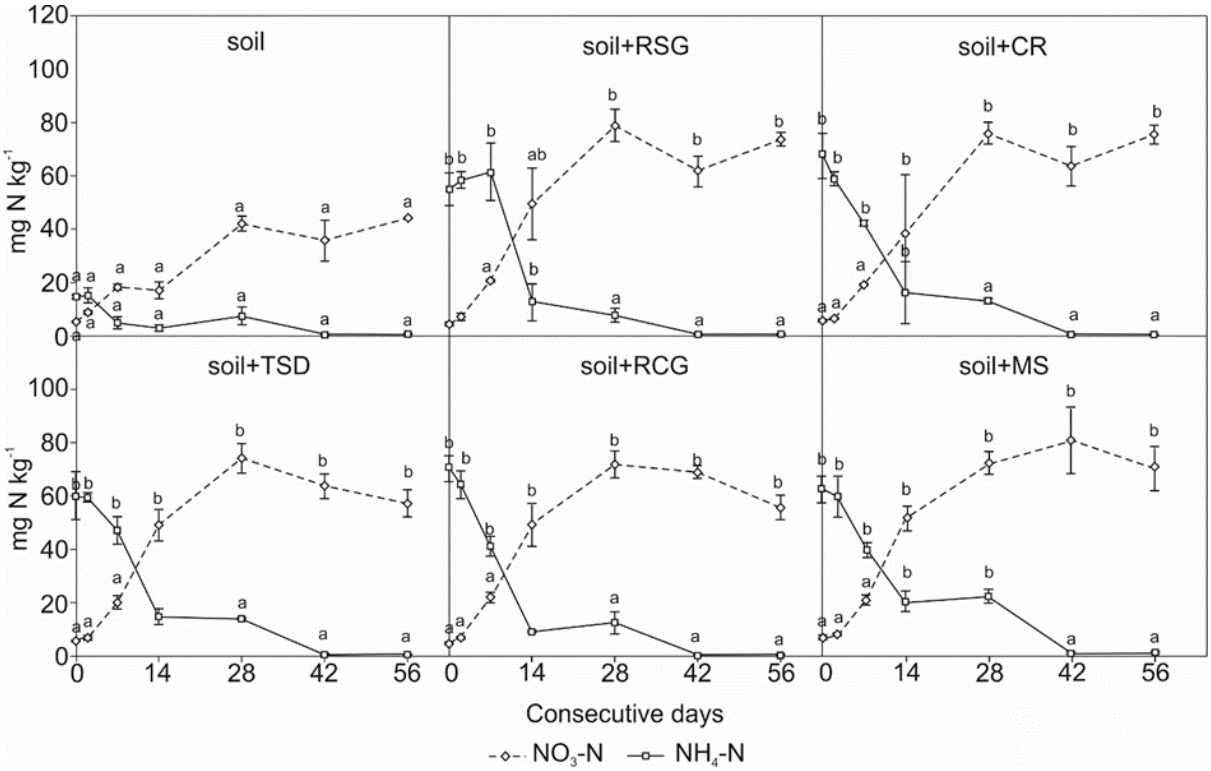

Figure 1. NO_3-N and NH_4-N dynamics during the incubation experiment (mean value ± standard deviation, where absent, bars fall within symbols) in the control (soil) and in soils treated with reed sweet-grass digestate (RSG), common reed digestate (CR), tufted sedge digestate (TSD), reed canary grass digestate (RCG) and maize digestate (MS). At the same time point, different letters indicate significant differences ($p < 0.05$) in means according to Tukey's test.

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MS, TSD and RCG, respectively (Table 3). The net mineralisation (N_m) of N added with digestates, which indicated the proportion of N immobilised in the soil, was equal to -19.86 %, -40.32 %, -41.57 % -58.79 % and -79.48 % for RSG, CR, MS, TSD and RCG, respectively. The nitrification conversion (N_c) at the end of incubation (Day 56) accounted for 53.39 % and 55.10 % of the TKN added in RSG and CR, respectively; and for 20.29 %, 21.15 % and 21.88 % in MS, RCG and TSD, respectively.

TKN in treated soils and in the control did not change with time. In unfertilised soil, TKN was stable throughout the incubation time and was equal to 1.05 ± 0.04 g kg^{-1}. In soil treated with digestates, TKN at Day 0 ranged from 1.05 ± 0.03 g kg^{-1} to 1.18 ± 0.02 g kg^{-1}, and at Day 56 from 1.06 ± 0.04 to 1.15 ± 0.05 g kg^{-1} (Figure 2). The amount of TKN was affected by the type of digestate applied ($F_{5,24} = 6.148$, $p < 0.001$). The TKN measured in soil fertilised with RSG, RCG and MS was similar to the amount in the control, while in soils treated with CR and TSD it was significantly higher ($p < 0.05$) than in the control.

Total organic carbon and C/N ratio

At the beginning of the experiment the TOC contents of all soils amended with digestates and the control were similar and in the range 13.74–15.28 g kg^{-1}. The type of digestate did not influence the TOC concentration in the soil throughout the incubation;

Table 2. Mean inorganic-N (g kg^{-1}) at different times during the incubation. Control = soil without amendments, RSG = soil fertilised with anaerobically digested reed sweet-grass, CR = soil fertilised with common reed digestate, TSD = soil fertilised with tufted sedge digestate, RCG = soil fertilised with reed canary grass digestate, MS = soil fertilised with maize digestate. Different appended letters indicate significant differences among treatments ($p < 0.05$) according to Tukey's test.

	Treatment					
Day	control	RSG	CR	TSD	RCG	MS
0	20.09a	61.09b	73.77b	64.84b	75.71b	67.89b
2	23.98a	67.31b	65.51b	65.77b	71.76b	66.75b
7	23.75a	83.25b	61.39c	66.41c	63.77c	60.06c
14	20.25a	63.47b	54.53b	62.90b	58.81b	69.67b
28	50.26a	88.41b	89.60b	87.53b	85.08b	92.25b
42	36.06a	62.74b	63.85b	63.18b	69.42b	79.27b
56	44.71a	74.61b	75.87b	56.77ab	56.18ab	69.31b

Table 3. Net mineralisation (N_{min}), net mineralisation of N added (N_m) and nitrification conversion of N added (N_c) in control and soils fertilised with digestates from anaerobic digestion of reed sweet-grass (RSG), common reed (CR), tufted sedge (TSD), reed canary grass (RCG) and maize (MS). n.a. = not analysed

soil	Net mineralisation (N_{min})	Net mineralisation of N added (N_m)	Nitrification conversion of N added (N_c)
	mg kg^{-1}	%	
control	24.67	n.a.	n.a.
RSG	13.53	-19.86	53.39
CR	2.10	-40.32	55.10
TSD	-8.07	-58.79	21.88
RCG	-19.53	-79.46	21.15
MS	1.42	-41.57	20.29

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however, the amount of TOC was affected by time ($F_{2,36} = 4.916$ $p < 0.05$). After 56 days the amount of TOC had decreased to 11.82–14.38 g kg^{-1}. A statistically significant drop in TOC was found for the control and for soil fertilised with CR (Figure 3).

C/N at Day 0 was equal to 15 in the control, 13 in soil fertilised with RSG, CR, TSD and MS, and 12 in soil amended with TSD. After 56 days of incubation, C/N had decreased to 13 in the control, 12 in soil fertilised with RSG and 11 in soil amended with CR, while in other treatments C/N did not change.

Electrical conductivity and pH

Soil electrical conductivity (EC) mirrored the pattern of NO_3-N in all fertilised soils and in the control. EC in the control was 55.3 ± 2.0 µS cm^{-1} at Day 0 and rose rapidly to 101.2 ± 2.9 µS cm^{-1} after a week of incubation and then to 167.4 µS cm^{-1} at the end of the experiment (Table 4). In soils fertilised with digestates, EC was significantly ($p < 0.05$) higher than in the control just after the fertiliser was added, then increased to 150 µS cm^{-1} after 7 days of incubation and to 330 µS cm^{-1} at Day 56. In all treated soils and in the control, the EC values were influenced by time, rising throughout the incubation (ANOVA $F_{6,84} = 936.86$, $p = 0.00$). The type of digestate did not influence either the EC value at the beginning of the incubation or the rate of increase throughout the experiment. However, EC was significantly lower in the control than in soil treated with digestates (ANOVA $F_{5,84} = 250.03$, $p = 0.00$) and, at Days 2, 28 and 56, EC was significantly lower in soil treated with MS than in soil fertilised with wetland plant digestates.

After addition of the digestates, pH increased slightly (not significantly, $p > 0.05$) in all of the treated soil samples, compared to the control (4.73 ± 0.02). The values for soils treated with digestate were ~0.3 units higher (Figure 4). A rapid decrease of pH in both unfertilised and fertilised soils was observed during the first 14 days of incubation, and after this period soil pH declined slowly to very similar values for all types of digestate ($p < 0.05$). After 28 days of incubation, the pH of soil treated with digestate was similar to that of the control ($p < 0.05$). The pH values measured on Days 14, 28, 42 and 56 were significantly ($p < 0.05$) lower than those found in the first week of incubation.

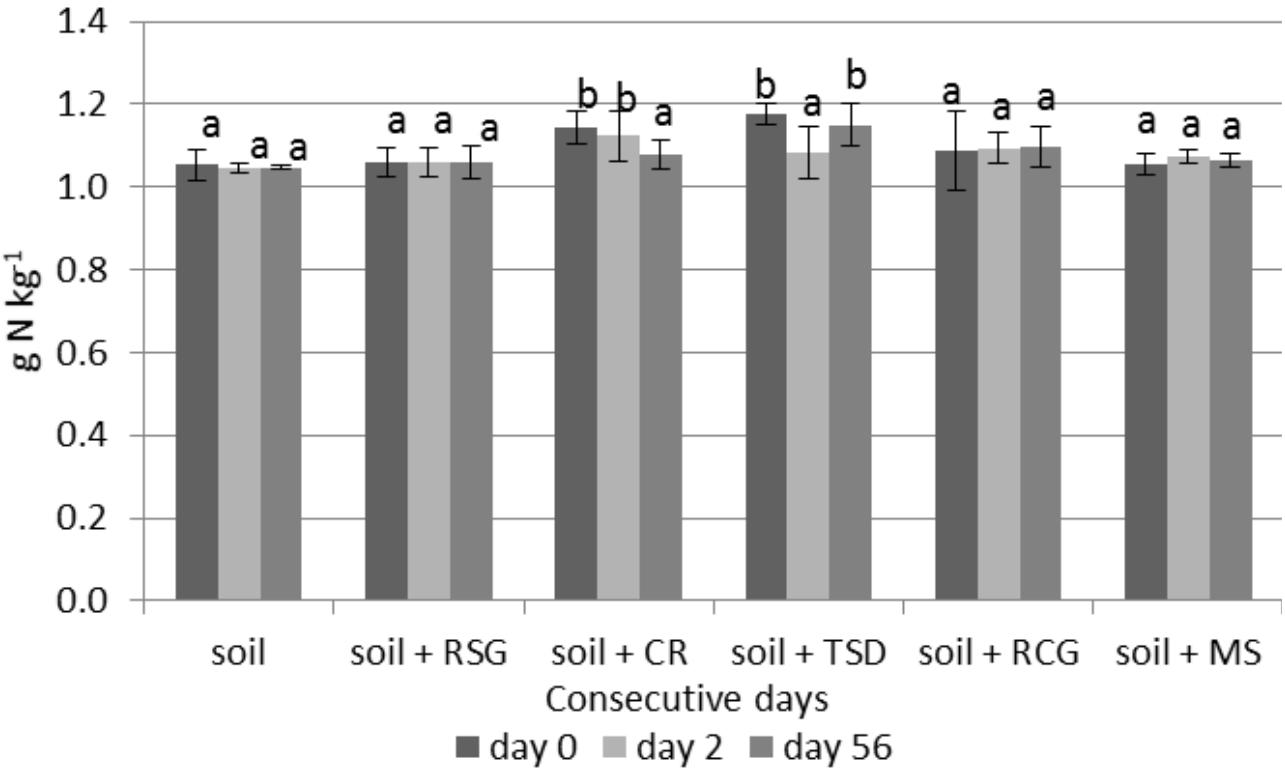

Figure 2. TKN content (mean value ± standard deviation) in the control (soil) and in soils treated with reed sweet-grass digestate (RSG), common reed digestate (CR), tufted sedge digestate (TSD), reed canary grass digestate (RCG) and maize digestate (MS). Different letters indicate significant differences at Days 0, 2 and 56 ($p < 0.05$) according to Tukey's test.

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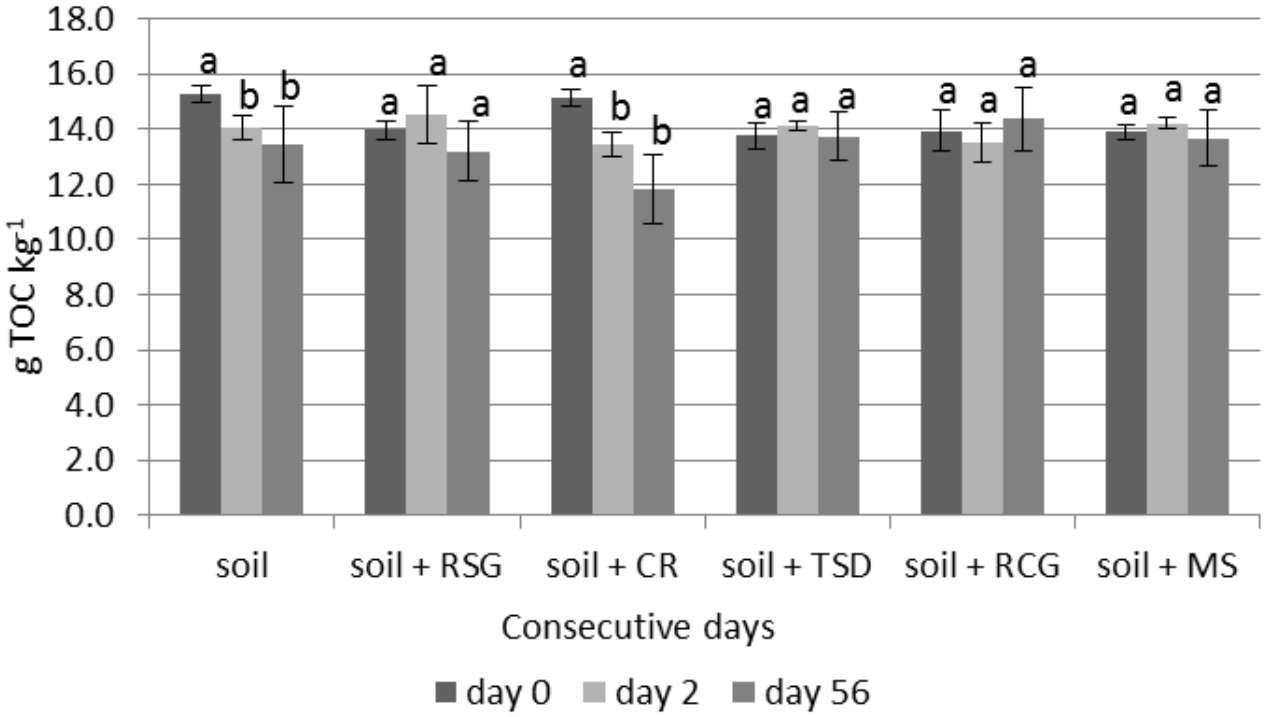

Figure 3. TOC content (mean value ± standard deviation) in the control (soil) and in soil treated with reed sweet-grass digestate (RSG), common reed digestate (CR), tufted sedge digestate (TSD), reed canary grass digestate (RCG) and maize digestate (MS). Different letters indicate significant differences in particular treatments ($p < 0.05$) according to Tukey's test.

Table 4. Mean EC (μS cm^{-1}) at different times during the incubation. Control = soil without amendments, RSG = soil fertilised with anaerobically digested reed sweet-grass, CR = soil fertilised with common reed digestate, TSD = soil fertilised with tufted sedge digestate, RCG = soil fertilised with reed canary grass digestate, MS = soil fertilised with maize digestate. Different appended letters indicate significant differences among treatments ($p < 0.05$) according to Tukey's test.

	Treatment					
Day	control	RSG	CR	TSD	RCG	MS
0	55.3a	96.6b	95.3b	92.3b	88.7b	80.1b
2	64.5a	109.6b	112.3b	110.0b	103.0be	92.4c
7	101.2a	159.6b	146.1b	149.9b	154.6b	148.3b
14	122.6a	257.2b	242.1b	251.0b	244.0b	216.5b
28	136.4a	308.0bd	238.9be	298.0b	298.7b	272.0c
42	154.1a	314.3b	293.3b	307.0b	300.7b	309.3b
56	167.4a	331.3b	335.0b	321.0bc	331.0b	285.7c

DISCUSSION

Digestate, as an AD by-product, is a promising source of N for use as a soil amendment to replace synthetic-N fertilisers (Sigurnjak *et al*. 2017). The TN is mostly conserved during the AD process (Field *et al*. 1984), possibly declining by 5–10 %, or even by up to 18 %, due to NH_4-N flux in the biogas stream, sedimentation of organic and inorganic matter, struvite formation, precipitation and retention in the digester (Schievano *et al*. 2011, Möller & Müller 2012). The organic-N in plant material is

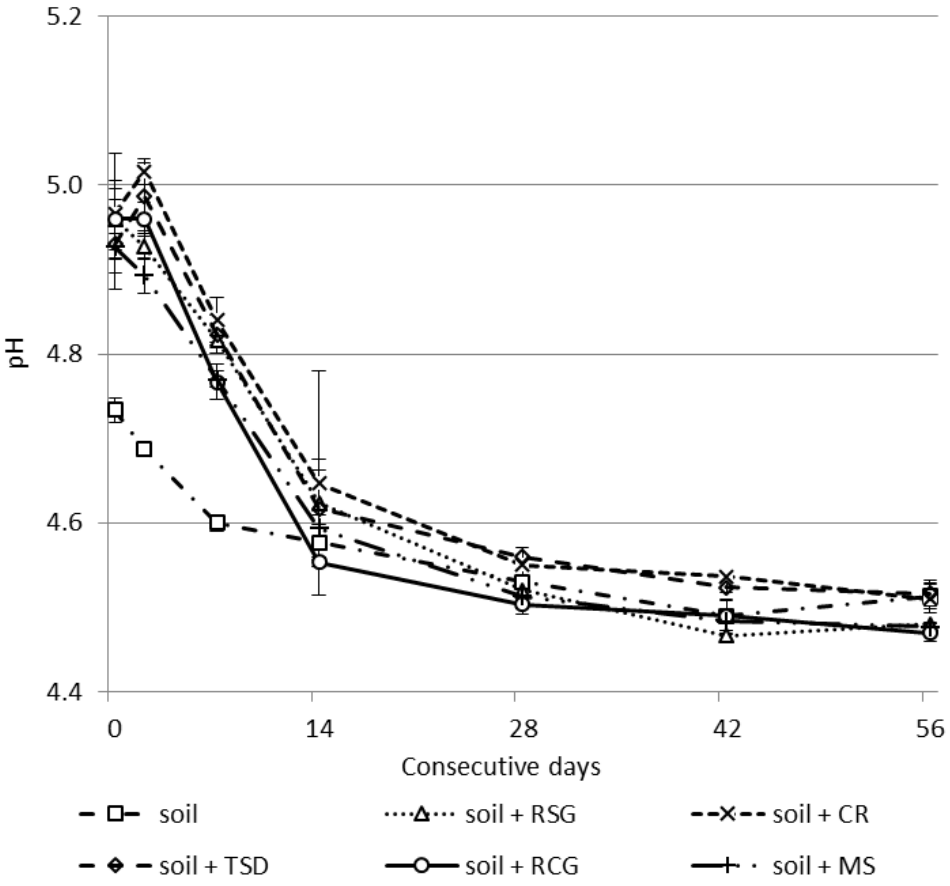

Figure 4. Soil pH dynamics (mean value ± standard deviation; where absent, bars fall within the symbols) during the incubation experiment, in the control (soil) and in soils treated with reed sweet-grass digestate (RSG), common reed digestate (CR), tufted sedge digestate (TSD), reed canary grass digestate (RCG) and maize digestate (MS).

mineralised to NH_4 during the AD process, so the digestates are characterised by high NH_4-N:TN ratio (Möller & Müller 2012). In the digestate-treated soil that we studied, 71–98 % of TKN was in the form of ammonium, which is in good agreement with results reported by Alburquerque *et al.* (2012c). A higher percentage of TKN as NH_4 could accelerate microbial biomass development and this could result in higher TKN content in soil fertilised with TSD and CR.

Digestate application triggers a range of soil processes including nitrification, immobilisation and emission. In the treated soil, a significant drop of NH_4-N content with a concomitant rise of NO_3-N occurred through nitrification of the NH_4-N applied (Grigatti *et al*. 2011, Alburquerque *et al*. 2012b, de la Fuente *et al*. 2013). The NO_3-N concentration exceeded the NH_4-N content at Day 3 in the control and at Day 9 in the treated soil, indicating rapid N transformation. A similar pattern of inorganic-N dynamics was observed by Rigby & Smith (2013) in sandy loam soil fertilised with digestate. Other processes which could lower the NH_4-N concentration are unlikely, because low clay content has been shown to result in rather low fixation of NH_4-N as a non-exchangeable form in soil, while mixing the digestate with soil during application prevented losses by ammonia volatilisation (de la Fuente *et al.* 2010). However, Cavalli *et al.* (2017) observed high rates of NH_4-N immobilisation in clay minerals in soil with low clay content. In our study, the inorganic-N losses at the end of incubation that resulted in negative N_m values can be explained to a rather small extent by immobilisation of N in clay minerals and mainly by immobilisation in microbial biomass due to the addition of organic C in the digestates, which results in an increase of microbial activity. The preferential microbial immobilisation of NH_4-N over NO_3-N is typical for soil mesocosms; however, if the NH_4-N is depleted or the NO_3-N content is much higher than the NH_4-N content, then the NO_3-N is assimilated by heterotrophic microorganisms (Burger & Jackson 2003). Despite the fact that up to 60 % of the organic C in AD

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substrates is turned into biogas and most of the C left behind is rather resistant to mineralisation, the labile organic C fraction influences the mineralisation-immobilisation turnover of N (Johansen *et al.* 2013). According to Alburquerque *et al.* (2012b), digestates are characterised by different biodegradability of organic matter, depending on their origin, and may thus differentially influence this process in the soil.

The NO_3-N recovery rate of 21 % for RCG, TSD and MS and 55 % for CR and RSG is in good agreement with results reported by Grigatti *et al.* (2011) and de la Fuente *et al.* (2013). Lower nitrification rate in soils fertilised with RCG and MS might be explained by lower pH values, since nitrification is sensitive to pH. Another possible explanation is the difference in concentration of easily degradable organic-N compounds in digestates derived from different species (de la Fuente *et al.* 2010). The pattern of inorganic-N transformation together with recovery rate suggest that digestate from the AD of biomass harvested from wetlands can be used successfully as a substitute for mineral N fertilisers (Alburquerque *et al.* 2012a). However, fast nitrification can be a limiting factor in field application of digestate due to the possibility of NO_3-N leaching (Goberna *et al.* 2011, Grigatti *et al.* 2011, Sigurnjak *et al.* 2017). Therefore, the application of digestate should follow the best agricultural management practices designed to reduce NO_3-N leaching. Appropriate timing of application to match the crop demand may reduce N losses from digestate used as fertiliser (Möller & Stinner 2009).

All treatments led to a significant increase of soil EC, to ~300 µS cm^{-1} at the end of incubation. This EC enhancement could be caused by the relatively high EC of digestates, ranging from 1305 µS cm^{-1} to 1760 µS cm^{-1} (Roj-Rojewski *et al.* 2018b). However, the increased EC was much lower than the threshold value for salinity (2000 µS cm^{-1}; Herrero & Perez-Coveta 2005). Therefore, from this point of view, digestate from wetland plants can be used as fertiliser on the uplands adjacent to riverine wetlands. However, de la Fuente *et al.* (2013) suggest that digestate application should be controlled to avoid excessive addition of salt to the soil, which may lead to its accumulation or leaching.

The slight increase in pH after digestate application could be related to the higher pH of the amendments, which was in the range 7.5–7.8. The rapid decline in pH over the next 14 days parallels the nitrification process which involves the release of H^+, giving an acidifying effect (Zhao *et al.* 2007). Such a pattern of pH changes in soils treated with pig slurry was reported de la Fuente *et al.* (2010). Loria & Sawyer (2005) observed that the initial increase of pH followed by a rapid decrease during the first 28 days of incubation was very similar for soils treated with raw and digested pig slurry, and smaller than for soil treated with mineral fertiliser, which could be caused by buffering from organic fertilisers. The optimal pH for crop production ranges from 5.5 to 7.0, while the pH in amended soils after 56 days of incubation was equal to 4.5 which suggests that application of the studied digestates, especially to sandy soils, should imply management for crop production similar to regimes based on NH_4-N mineral fertilisers (Loria & Sawyer 2005). This is in good agreement with de la Fuente *et al.* (2013), who reported that the suitability of digestate solid fraction for crop production may be limited because of its negative effect on soil pH.

On the basis of harvested biomass yield and N content, we can calculate the area which might be fertilised with the digestate obtained from biomass harvested from 1 ha of wetland. The biomass of the studied species may vary significantly. The dry biomass yield of reed sweet-grass ranges from 10 to 15 t ha^{-1} (Jankowska-Huflejt & Domański 2008), and the variation in reed is even higher with a range of 3.1 t ha^{-1} to 41 t ha^{-1} depending on habitat (Brix *et al.* 2001). However, Jankowska-Huflejt & Domański (2008) reported 12–30 t ha^{-1} of aboveground dry biomass, while Köbbing *et al.* (2013) reviewed 23 literature sources from 22 countries and reported that harvested reed dry biomass ranged from 5 to 20 t ha^{-1}. The yield depends mainly on climate, soil, water and nutrients, but also on time of harvesting (Mulkeen *et al.* 2017). The dry biomass production of common reed in the Biebrza River Wetland, located close to the Narew River, depends on the situation of the stand and varies from 3 t ha^{-1} in central zones of the river valley to 28 t ha^{-1} in the narrow belt close to the river, which is rich in nutrients due to regular flooding (Szczepański *et al.* 1993). A high yield of 10–15 t ha^{-1} can also be obtained by harvesting reed canary grass (Jankowska-Huflejt & Domański 2008). The least productive species is tufted sedge with aboveground dry biomass ranging from 3.7 t ha^{-1} to almost 10 t ha^{-1} (Stelmaszczyk *et al.* 2015). For further calculations, the average dry biomass yield for each species was adopted.

In the wetland plants studied, dry mass N content followed the sequence: RCG (26.1 ± 1.6 g kg^{-1}) > CR (21.1 ± 1.3 g kg^{-1}) > TSD (16.5 ± 2.6 g kg^{-1}) > RSG (12.4 ± 0.6 g kg^{-1}). The results of our study are in good agreement with data from the literature. The dry mass TN content is highly variable and in aboveground biomass of reed sweet-grass ranges from 14 to 23 g kg^{-1} depending on time of harvest (Sundblad & Robertson 1988), in reed it ranges from

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2 to 25 g kg^{-1} (Köbbing *et al.* 2013, Mulkeen *et al.* 2017), in reed canary grass it is 14.6 g kg^{-1} (Oleszek *et al.* 2014), and in tufted sedge it can reach 14.4 g kg^{-1} (Stelmaszczyk *et al.* 2015).

If we assume that TN is conserved during the AD process and that the statutory N application rate of 170 kg ha^{-1} is implemented on the arable land in the form of organic fertilisers, we can calculate the area that could be fertilised with the digestate obtained from biomass harvested from 1 ha of average-productivity wetland. The digestate could meet the N fertiliser requirement on 0.7 ha for TSD, 0.9 ha for RSG, 1.9 ha for RCG and 2.0 ha for CR. These results suggest that, because of its high productivity, common reed is the most suitable plant for AD processing if not only energy production but also fertilisation is taken into account. This means that, by using harvested biomass from natural wetlands or rewetted areas for biogas production, farmers could acquire not only 'clean' energy but also good quality cheap fertiliser. This would benefit the fragile wetland ecosystem by partially closing the loop of the N cycle between wetlands and adjacent arable land. Such management of wet areas may change their perceived worth in local communities from unproductive areas that block progress to valuable sources of energy and nutrients for fertilisation.

CONCLUSIONS

Our study demonstrates that digestates derived from wetland plants can provide a large amount of NH_4-N to the soil, regardless of the plant species used as AD feedstock. The overall pattern of N dynamics is similar in soils fertilised with digestates from wetland plant species and from maize. However, at landscape scale, the incorporation of digestate from wetland plants harvested in the swampy river valley into the agricultural soils upslope seems a much better option environmentally than fertilisation with digestate obtained from a dedicated crop such as maize, because it reduces the surplus of N in the landscape. Also, AD gives more environmental advantages than other uses of harvested wetland biomass because it produces not only digestate (as a by-product) but also biogas from which electricity and heat can be generated without burning fossil fuels.

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