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Advanced Processing of Food Waste Based Digestate for Mitigating Nitrogen Losses in a Winter Wheat Crop

Antonio R. Sánchez-Rodríguez, Alison M. Carswell, Rory Shaw, John Hunt, Karen Saunders, Joseph Cotton, Dave R. Chadwick, Davey L. Jones and Tom H. Misselbrook

The anaerobic digestion of food waste converts waste products into “green” energy. Additionally, the secondary product from this process is a nutrient-rich digestate, which could provide a viable alternative to synthetically-produced fertilizers. However, like fertilizers, digestate applied to agricultural land can be susceptible to both ammonia (NH₃) and nitrous oxide (N₂O) losses, having negative environmental impacts, and reducing the amount of N available for crop uptake. Our main aim was to assess potential methods for mitigating N losses from digestate applied to a winter wheat crop and subsequent impact on yield. Plot experiments were conducted at two UK sites, England (North Wyke-NW) and Wales (Henfaes-HF), to assess NH₃ and N₂O losses, yield and N offtake following a single band-spread digestate application. Treatments examined were digestate (D), acidified-digestate (AD), digestate with the nitrification inhibitor DMPP (D+NI), AD with DMPP (AD+NI), and a zero-N control (C). Ammonium nitrate (NH₄NO₃) fertilizer N response plots (from 75 to 300 kg N ha⁻¹) were included to compare yields with the organic N source. Across both sites, cumulative NH₃-N losses were 27.6% from D and D+NI plots and 1.5% for AD and AD+NI of the total N applied, a significant reduction of 95% with acidification. Cumulative N₂O losses varied between 0.13 and 0.35% of the total N applied and were reduced by 50% with the use of DMPP although the differences were not significant. Grain yields for the digestate treatments were 7.52–9.21 and 7.23–9.23 t DM ha⁻¹ at HF and NW, respectively. Yields were greater from the plots receiving acidified-digestate relative to the non-acidified treatments but the differences were not significant. The yields obtained for the digestate treatments ranged between 84.2% (D+NI) and 103.6% (D) of the yields produced by the same N rate from an inorganic source at HF. Advanced processing of digestate reduced N losses providing an environmentally sound option for N management.

Keywords: ammonia volatilization, greenhouse gas emissions, N₂O, nitrification inhibitors, acidification, digestate
INTRODUCTION

During the last few decades, the interest in anaerobic digestion in the European Union (EU) has increased due to the development of regulations and guidelines that encourage the production of renewable energy to benefit the environment (Siebert et al., 2008; EU, 2009; BSI, 2010). Anaerobic digestion plants generate biogas (rich in methane), a source of “green” energy, and a liquid by-product known as digestate, with a high potential as fertilizer or soil conditioner depending on its nutrient content (Nkoa, 2014). The EU has promoted nutrient recovery as part of the circular economy (EU, 2014) encouraging digestate to be valued as an alternative to inorganic and non-renewable fertilizers in agriculture, as a potential source of income rather than a waste or by-product (Alburquerque et al., 2012a,b; Nkoa, 2014; Kataki et al., 2017).

The main feedstocks for biogas plants are energy crops, animal manures, and other organic wastes (Lukehurst et al., 2010) depending on what is locally available. In some countries of the EU, including the UK, anaerobic digestion is the recommended technology for sanitizing food waste from supermarkets, catering, and kitchen waste (Lukehurst et al., 2010), and their treatment through anaerobic digestion is increasing (Styles et al., 2016). Nevertheless, there is a lack of evidence for the agronomic and environmental effects of the application of food waste derived digestate to agricultural land.

Anaerobic digestion modifies the former properties of the feedstocks, affecting N cycling and bioavailability once the digestate is applied to the soil as a source of nutrients for crops. The enhanced microbial degradation of organic matter and emission of carbon (C), particularly as methane, results in an increase in the proportion of total N that is more readily plant available (i.e., in increase in the ratio of ammonium-N (NH$_4^+$-N) to total N, typically to >70%), a decrease in the C:N ratio and a lower organic matter and dry matter (DM) content (Webb and Hawkes, 1985; Möller et al., 2008; Tampio et al., 2016). Anaerobic digestion can significantly reduce greenhouse gas and odor emissions (if fugitive emissions are minimized) in comparison with the feedstock (Masse et al., 2011; Battini et al., 2014), and produces a more sanitized product when the feedstock is manure (Orzi et al., 2015). However, the increase in pH and NH$_4^+$-N content through anaerobic digestion enhance the polluting potential of the digestate during storage (Sommer and Husted, 1995) and following land spreading (Möller, 2015). The main concerns regarding application of digestate and other organic wastes to agricultural land are emissions of N to the environment through ammonia (NH$_3$) volatilization, nitrate (NO$_3^-$) leaching and greenhouse gas emissions as nitrous oxide (N$_2$O), with associated impacts on air and water quality, ecosystem functioning and human health (Galloway et al., 2003).

Tiwary et al. (2015) reported that 35–65% of the total N applied in digestate can be lost through NH$_3$ volatilization if the digestate is surface broadcast. Potential methods to reduce NH$_3$ volatilization include the rapid incorporation of manures and digestates into the soil after application (Möller et al., 2008; Tiwary et al., 2015), soil injection (Riva et al., 2016), band-spreading (Nicholson et al., 2017), and acidification of slurries (Fanguiero et al., 2015a).

Nitrous oxide emissions following digestate application to land are thought to be lower than those emitted from the undigested material because most of the available C has been converted to biogas prior to land application. However, there are contradictory reports from the literature (Möller, 2015) suggesting that emissions are related to the feedstocks and soil properties to which they are applied, e.g., soil organic matter content, soil texture, water content, and aeration (Chantigny et al., 2009; Eickenscheidt et al., 2014). Reported N losses as N$_2$O emissions following the application of food-based digestate vary from 0.45% (Nicholson et al., 2017) to 4–10% (Tiwary et al., 2015) of the total N applied. A method to reduce N$_2$O emissions from manure applications, which may be equally applicable to digestates is the use of nitrification inhibitors (NI), such as 3,4-Dimethylpyrazole phosphate (DMPP) (Owusu-Twum et al., 2017), that delay the process in which NH$_4^+$ transforms into NO$_3^-$. Nitrate is a readily mobile form of N, which can be lost by leaching, therefore, keeping N in the form of NH$_4^+$ (less-mobile) could prevent NO$_3^-$ leaching while minimizing N$_2$O losses (Subbarao et al., 2006; MarkFoged et al., 2011).

The main objective of this study was to compare the efficiency of different N loss mitigation strategies (acidification, use of a nitrification inhibitor, and the combination of both) to reduce N losses (NH$_3$ volatilization and N$_2$O emissions) and enhance the value of food waste based digestate as a source of N for a winter wheat crop. Our hypothesis was that the acidification of the digestate and the use of a nitrification inhibitor (i.e., DMPP) would decrease N losses in relation to untreated digestate, improving the N use efficiency for crop yield and thereby the potential of digestate as an alternative to an inorganic fertilizer N source.

MATERIALS AND METHODS

Site Description and Experimental Design

Two field experiments were conducted on a winter wheat crop over the 2016-2017 UK growing season. The first site was at the Henfaes Research Station (HF), in Abergywregyn, North Wales (53°14′21.3″N, 4°05′30.3″W; 10 m above sea level). The second site was at Rothamsted Research North Wyke (NW), in Devon, South West England (50°79′39.8″N, 3°95′25.1″E; 180 m above sea level). The former crop was barley at HF and grassland at NW. Both sites have a temperate climate with average annual rainfall of 1,060 and 1,107 mm, respectively. The soil at HF is a free-draining Eutric Cambisol with a sandy clay loam texture (IUSS, 2015). Five representative soil samples were collected from each field site to a depth of 15 cm. Each soil sample was then crumbled by hand, vegetation, roots, and stones manually removed and the soil thoroughly mixed prior to analysis. The main soil characteristics are shown in Table 1.

*Triticum aestivum* (var. KWS Siskin) was drilled on the 10th October 2016 at both sites with a row spacing of 0.1 m. Prior to this, the fields were plowed to 15 cm depth and limed to increase...
the soil pH. Phosphorus (P, as Ca(H₂PO₄)) and potassium (K, as KCl) were applied during the same week of sowing. Kieserite (MgSO₄ ⋅ H₂O) was applied in March at both sites. Application rates were based on routine soil analyses and national fertilizer guidelines (DEFRA, 2010), so that these elements were non-limiting. Herbicides at both sites, and insecticides and fungicides only at NW were also applied according to manufacturers’ recommendations. See Table S1 for additional information.

A randomized complete block design was established at each site with one replication in each block equalling five replications per treatment (n = 5), with plot size 14 × 1.2 m at HF and 9 × 2 m at NW. There were four “digestate treatments” and a control:

1. control (C): zero-N, no digestate or fertilizer N applied
2. digestate (D);
3. digestate + the nitrification inhibitor 3,4-dimethylpyrazole phosphate (D+NI): DMPP (21 ha⁻¹) was added to the digestate and gently stirred before application;
4. acidified digestate (AD): digestate previously acidified in 1 m³ tanks;
5. acidified digestate with nitrification inhibitor (AD+NI): DMPP (21 ha⁻¹) was added to acidified digestate and gently stirred before application.

The target application rate was 190 kg N ha⁻¹ as digestate, although actual application rate achieved in the field varied (Table 2). The digestate was band-spread parallel with crop rows (30 cm between bands) at a rate equivalent to 40 m³ ha⁻¹ using 201 capacity watering cans on April 19th 2017 at HF and March 20th 2017 at NW, at the start of stem elongation and never after early May, according to DEFRA (2010). The digestate remained in bands in the “digestate treatments” at NW but not at HF because of the lower DM content. The plots were divided into two different areas: (1) the harvest area, which was used to determine grain yields and plant production; and (2) the sampling area, which was used for periodic soil sampling, NH₃ volatilization measurements (wind tunnels) and daily N₂O emissions (manual or automatic chambers, Table 2). At HF, NH₃ emission measurements were made on the main plots, whereas at NW separate “mini-plots” (2 × 0.5 m) were established for these measurements at the prevailing wind (south westerly) edge of the trial site.

Additionally, to be able to calculate the fertilizer replacement rate of the N mitigation digestate treatments, N response plots were included at both sites. Ammonium nitrate (NH₄NO₃) was applied at four different rates: 75, 150, 225, and 300 kg N ha⁻¹ split into three applications between March and April 2017 according to the suggestions by DEFRA (2010) for winter wheat. The N response plots were 6.5 × 1.2 m at HF, where they were included in the randomized block design, and 4.5 × 2 m at NW, where they were established in a separate part of the field.Nitrogen response plots were for yield measurement only with no soil or gaseous emission sampling.

Table 3 gives the main properties of the anaerobic digestate used in the field experiments from six (HF) and 12 (NW) digestate samples. The digestate, based on food waste and without separating solid and liquid fractions, was provided from local anaerobic digestion plants. Half of the digestate used at each site was acidified, to a target pH of 5.5, with concentrated H₂SO₄ before application. Approximately 11 of concentrated H₂SO₄ was added in total per 100 l of digestate. The pH of the digestate at application was determined in a 1:6 (v/v) fresh digestate:distilled water suspension and was lower for the acidified digestate than in the non-acidified digestate at both sites, as expected (Table 3), although the reduction in pH to <3 for the NW site was greater than anticipated based on previous laboratory tests.

### Soil Sampling

During the experiment, soil was sampled from the sampling area of each plot three times per week for the first 2 weeks after digestate application, two times per week for the next 2 weeks, followed by weekly sampling thereafter. Subsequently, soil samples were taken once per month until the end of the experiment. On each occasion, eight soil samples were taken per plot to 15 cm depth and pooled to provide one representative sample per plot. At NW, soil was sampled proportionally from within and between the digestate bands. At HF, soil was sampled randomly, as there were no distinct digestate bands. Soil samples were stored at 4°C and in the dark prior to analyses. Soil moisture, pH, EC, NH₄⁺, and NO₃⁻ were determined as detailed previously.

### Analytical Methods

#### Chemical Properties

Soil pH and electrical conductivity (EC) were determined in a 1:2.5 (w/v) soil:distilled water suspension with standard electrodes using a Model 209 pH meter (Hanna Instruments Ltd., Leighton Buzzard, UK) and a Jenway 4520 conductivity meter (Cole-Palmer Ltd., Stone, UK). Total soil C and N were determined using a TruSpec® analyser (Leco Corp., St Joseph, MI) and ground oven-dried soil (105°C, 24 h). A soil subsample was taken to determine soil moisture and another for mineral N extractions: a 0.5 M K₂SO₄ solution was used in a 1:5...
and NO\textsuperscript{−} following centrifugation (10,000 g, 10 min) and then centrifuging at 10,000 g for 10 min. The supernatant was stored at −20°C until analyses. Total dissolved organic C (DOC) and total dissolved N (TDN) in the extracts were measured using a Multi N/C 2100/2100 analyser (AnalytikJena AG, Jena, Germany). Dissolved organic N (DON) was calculated by subtracting NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} from the TDN value. Ammonium in the extract was determined colorimetrically using the salicylate method of Mulvaney (1996) and NO\textsubscript{3}\textsuperscript{−} following the salicylate method of Miranda et al. (2001) in an Epoch\textsuperscript{®} microplate spectrophotometer (Bio Tek Instruments Inc., Winooski, VT). Mineralisable N was determined after anaerobic incubation according to Keeney (1982) using 5 g of soil and calculating the differences in NH\textsubscript{4}\textsuperscript{+} between the initial concentrations and the concentrations after 7 days of anaerobic incubation. Acetic acid extractable P was used as a proxy for plant-available P, determined after extracting the soil with 0.5 M acetic acid (1:5 w/v, 200 rev min\textsuperscript{−}1 for 1 h) by the molybdate blue method (Murphy and Riley, 1962) following centrifugation (10,000 g, 10 min).

Total N, and NO\textsubscript{3}\textsuperscript{−}-N in the digestates were determined as previously described, and NH\textsubscript{4}\textsuperscript{+}-N was significantly higher in the anaerobic digestate for both sites. A digestate sub-sample was oven-dried at 105°C for 24 h and ground to pass 1 mm sieve

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### TABLE 2 | Plots dimensions, N application rates, and measurements conducted at the Henfaes (HF) and North Wyke (NW) field experiments.

<table>
<thead>
<tr>
<th>Plot</th>
<th>Size (m\textsuperscript{2})</th>
<th>N applied\textsuperscript{a} (kg N ha\textsuperscript{−}1)</th>
<th>Measurements</th>
<th>Size (m\textsuperscript{2})</th>
<th>N applied\textsuperscript{a} (kg N ha\textsuperscript{−}1)</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control plots</td>
<td>14 × 1.2</td>
<td>0</td>
<td>Yield, plant production, N content, N offtake, NUE\textsuperscript{D}</td>
<td>9 × 2</td>
<td>0</td>
<td>Yield, plant production, N content, N offtake, NUE\textsuperscript{D}</td>
</tr>
<tr>
<td>Harvest area</td>
<td>6.5 × 1.2</td>
<td>0</td>
<td>Soil NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{3}\textsuperscript{−}, soil pH, and EC\textsubscript{C}, N\textsubscript{2}O</td>
<td>4.5 × 2</td>
<td>0</td>
<td>Soil NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{3}\textsuperscript{−}, soil pH, and EC\textsubscript{C}, N\textsubscript{2}O</td>
</tr>
<tr>
<td>Digestate plots</td>
<td>14 × 1.2</td>
<td>132 (D, D+Ni), 176 (AD, AD+Ni)</td>
<td>Yield, plant production, N content and offtake, NUE\textsuperscript{D}</td>
<td>9 × 2</td>
<td>177 (D, D+Ni), 217 (AD, AD+Ni)</td>
<td>Yield, plant production, N content and offtake, NUE\textsuperscript{D}</td>
</tr>
<tr>
<td>Harvest area</td>
<td>7.5 × 1.2</td>
<td>132 (D, D+Ni), 176 (AD, AD+Ni)</td>
<td>Soil NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{3}\textsuperscript{−}, soil pH and EC\textsubscript{C}, NH\textsubscript{3}, N\textsubscript{2}O</td>
<td>4.5 × 2</td>
<td>177 (D, D+Ni), 217 (AD, AD+Ni)</td>
<td>Soil NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{3}\textsuperscript{−}, soil pH and EC\textsubscript{C}, N\textsubscript{2}O</td>
</tr>
<tr>
<td>N response plots</td>
<td>7.5 × 1.2</td>
<td>75/150/225/300</td>
<td>Yield, plant production, N content and offtake, NUE\textsuperscript{D}</td>
<td>4.5 × 2</td>
<td>75/150/225/300</td>
<td>Yield, plant production, N content and offtake, NUE\textsuperscript{D}</td>
</tr>
<tr>
<td>Mini-plots</td>
<td>2 × 0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest area</td>
<td>7.5 × 1.2</td>
<td>177 (D, D+Ni), 217 (AD, AD+Ni)</td>
<td>NH\textsubscript{3}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling area</td>
<td>2 × 0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}N applied, Control; D, digestate; D+Ni, digestate plus nitrification inhibitors; AD, acidified digestate; AD+Ni, acidified digestate plus nitrification inhibitors.

\textsuperscript{D}NUE, nitrogen use efficiency.

\textsuperscript{C}EC, electrical conductivity.

### TABLE 3 | Properties of the digestate (D) and acidified digestate (AD) used at Henfaes (HF, n = 3, mean ± standard error) and North Wyke (NW, n = 6; mean ± standard error) expressed on a fresh weight basis.

<table>
<thead>
<tr>
<th>Test</th>
<th>HF</th>
<th>NW</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:8 w/v)</td>
<td>8.24 ± 0.01</td>
<td>8.05 ± 0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>3.08 ± 0.19</td>
<td>7.52 ± 0.08</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total N (g kg\textsuperscript{−}1)</td>
<td>3.30 ± 0.12</td>
<td>4.43 ± 0.06</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+}-N (%) of N</td>
<td>90.3 ± 2.7</td>
<td>81.7 ± 1.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NO\textsubscript{3}\textsuperscript{−}-N (mg kg\textsuperscript{−}1)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>na</td>
</tr>
<tr>
<td>Total P (g kg\textsuperscript{−}1)</td>
<td>0.56 ± 0.02</td>
<td>0.76 ± 0.01</td>
<td>1.05 ± 0.01</td>
</tr>
<tr>
<td>Total K (g kg\textsuperscript{−}1)</td>
<td>1.55 ± 0.01</td>
<td>1.07 ± 0.01</td>
<td>0.958</td>
</tr>
</tbody>
</table>

P is the P-value of the ANOVA for all the properties except for pH and dry matter for NW that is the P-value of the Kruskal-Wallis non-parametric ANOVA. na, not-applicable.
to determine the dry matter (DM) content. Dry matter content was greater in the digestate than in the acidified digestate, and greater at NW than at HF (mean values, Table 3). A sample of each digestate was digested with concentration hydrochloric and nitric acid (aqua-regia) to analyse mineral elements by ICP-OES / ICP-MS as detailed in EPA (1996); the acidified digestate had a significantly higher content in Mg at HF and in S at both sites but a lower content in Zn at NW (Table S2).

Ammonia Volatilization
Ammonia volatilization measurements were made using a system of small wind tunnels as described by Messelbrook et al. (2005). One wind tunnel was placed on each of the “digestate plots” of the four first blocks at HF and on each of the mini-plots at NW directly after the application of the “digestate treatments” (n = 4 for each treatment). Ammonia concentrations of the inlet and outlet air of each wind tunnel were determined using 0.02 M H$_3$PO$_4$ acid traps (100 ml) changed every day, except for the first day when higher volatilization rates were expected they were changed twice at HF and three times at NW. After each sampling, the acid trap samples were made up to 100 ml with distilled water in the laboratory and a subsample was frozen before analysis for NH$_3$-N as described previously. Ammonia fluxes ($F_{NH3}$, µg m$^{-2}$ s$^{-1}$) were calculated according to equation 1:

$$F_{NH3} = \nu (C_o - C_i)/At$$

where $C_o$ and $C_i$ are the outlet and inlet concentrations, respectively, $\nu$ is the air volume (m$^3$) drawn through the wind tunnel over the sampling period (t, s), and A the area covered by the wind tunnel (m$^2$).

Cumulative NH$_3$ emissions over the 7 day measurement period were derived by summing the flux from each sampling time. Total N lost through NH$_3$ volatilization was expressed as a percentage of the total N applied for each treatment to normalize for the different N application rates at the two sites.

Nitrous Oxide Emissions
Nitrous oxide emissions were measured with a combination of static manual and automated chambers at HF and only manual chambers at NW. Specifically, three replicate plots with one automated chamber (0.5 × 0.5 × 0.2 m) per plot were used for the “digestate” treatment plots at HF, with one manual static chamber (0.5 × 0.5 × 0.3 m) per plot for three control plots (i.e., n = 3 per treatment at HF). At NW, one manual static chamber (0.5 × 0.5 × 0.3 m) was used on each replicate plot for all treatments (n = 5 per treatment). The automatic chambers at HF were linked to an Isotopic N$_2$O Analyser (Los Gatos Research Inc., San Jose, CA, USA) for measurement of N$_2$O concentration. All chambers were programmed to close sequentially using pneumatic actuators, for 30 min for gas sampling, resulting in four measurements per chamber per day. Gas was sampled from the chambers via a sampling port at a rate of 11 min$^{-1}$, and to avoid a negative pressure, the chambers allowed ambient air entry via an air inlet hole of the same diameter as the sampling one, i.e. these were through-flow chambers. Gas samples were delivered to an Isotopic N$_2$O Analyser via 0.17 mm internal diameter PFA tubing, with the same length for all chambers. Nitrous oxide concentrations were recorded at 0.1 Hz during the 30 min chamber closure. N$_2$O concentration data for the first 0.5 min was discarded from calculations to account for the dead volume in the sample lines. Every four chambers, a standard (1.5 ppm N$_2$O) was introduced into the analyser for calibration.

Hourly N$_2$O fluxes (µg N$_2$O-N m$^{-2}$ h$^{-1}$) were calculated using linear regression, with the assumption of linearity for manual and automatic chambers. Calculations for the automatic chamber determinations were made using the lm() function in R (version 3.3.2., R Core Team 2016). The manual chamber N$_2$O emissions ($F_{N2O}$) were calculated as described by de Klein and Harvey (2012); (Excel, Office 2016) using Equation (2):

$$F_{N2O} = H × (C_i − C_0)/t$$

where $H$ is the ratio of chamber volume to soil surface area (l$^3$ to l$^{-2}$), $C_i$ is the concentration of N$_2$O within the chamber at the time (t) of sampling and $C_0$ is the N$_2$O concentration measured at 0 min, measured after the chamber had been sealed. Cumulative N$_2$O emissions were calculated for each plot using the area under a curve function “cumtrapz()” from the “pracma” package (Hans Werner Borchers; R Core Team, 2016). Finally, total N lost as N$_2$O was expressed as the percentage of total N applied in each treatment after subtracting the cumulative N$_2$O emissions from the control plots.

Grain, Plant Production, and Nitrogen Use Efficiency
Grain and plant production were determined from the “harvest area” of each plot at the end of the experiment (8 and 15th August 2017 at NW and HF, respectively). At HF, wheat plants from three 0.4 × 0.4 m quadrats were harvested 2 cm above the ground and grain and straw were separated by hand and weighed. At NW,
a Sampo small-plot combine harvester was used to harvest the wheat, separating the grain and straw, which were weighed. A sub-sample from each plot was used to determine grain and straw moisture. Total N was analyzed using a TruSpec® analyser (Leco Corp., St Joseph, MI) from ground oven-dried plant tissue (80°C, 24 h); N offtake by the total crop was calculated by multiplying the N content of the grain and the straw by the grain and straw yield, respectively. Thousand-grain weight (TGW) was also determined by weighing 1,000 oven-dried grains. Grain yield, straw yield, respectively. Thousand-grain weight (TGW) was also determined by weighing 1,000 oven-dried grains. Grain yield, straw yield and TGW are reported at 85% dry matter.

Nitrogen Use Efficiency of the crop (total for grain and straw, \( \text{NUE}_c \)) and grain (\( \text{NUE}_g \)) were calculated according to Equations (3, 4), respectively:

\[
\text{NUE}_c = (N_t - N_c)/N_{\text{applied}} \times 100
\]

where \( N_t \) is the crop N offtake from N (digestate or NH\(_4\)NO\(_3\)) treatment plots, \( N_c \) is the mean crop N offtake from the control plots and \( N_{\text{applied}} \) is the N fertilizer applied to the plots. All units are in kg N ha\(^{-1}\).

\[
\text{NUE}_g = (N_{gt} - N_{c})/N_{\text{applied}} \times 100
\]

where \( N_{gt} \) is the grain N offtake from N (digestate or NH\(_4\)NO\(_3\)) treatment plots, \( N_{c} \) is the mean grain N offtake from the C plots and \( N_{\text{applied}} \) is the N fertilizer applied to the plots. All units are kg N ha\(^{-1}\).

Statistical Analyses
A factorial analysis of variance (ANOVA) with two factors (site: HF and NW; and treatment: C, D, D+NI, AD, AD+NI) and a blocking factor was performed for cumulative NH\(_3\) and N\(_2\)O losses (expressed as % of the total N applied), grain and straw yield, N offtake (grain, straw and total), TGW, NUE\(_c\), and NUE\(_g\). Tukey’s post-hoc was used to detect differences between sites and treatments. The t-test was performed to examine variation between the different properties of the digestate and acidified digestate used at both sites, except for pH and DM at NW where a Kruskal-Wallis non-parametric ANOVA was used. One-way ANOVA was used to compare NUE\(_c\), NUE\(_g\), grain yields, and plant production for the different “digestate treatments” and fertilizer N rates at HF. Cumulative NH\(_3\) losses, N offtake by straw, and plant production were log transformed to ensure the requirements for ANOVA. Statistical significance is defined as \( p < 0.05 \). In addition, linear (without including the highest dose) and quadratic regressions were derived for yield and total crop production for the fertilizer N response plots (0 to 300 kg N ha\(^{-1}\)) to calculate the fertilizer N replacement value of the different digestate treatments. All statistical analyses were performed using SPSS v22.0 (IBM Corp., Armonk, NY).

RESULTS
Soil Analyses
Soil pH, total C, total N, C:N ratio and mineralizable N were higher in HF than NW (Table 1). Changes in soil pH, \( \text{NH}_3^+ \), and \( \text{NO}_3^- \) during the experiment are presented in Figure 1 for both sites. Soil pH decreased following addition of the acidified digestate treatments (AD and AD+NI were between 5.2–6.3 at HF and AD+NI between 4.6–5.4 at NW) relative to the non-acidified treatments (C, D, and D+NI; 6.0–6.8 at HF, and C and D between 5.0–6.0 at NW; Figures 1A,B). This effect was observed a few days after digestate application and pH remained lower until harvest at both sites, reaching maximum difference 1 month before harvest (around 1.0 pH unit). The application of the digestate also led to changes in soil EC, with the greatest values for AD and AD+NI, followed by D and D+NI and, finally, by C (Figure S2).

Peaks in soil \( \text{NH}_3^+ \) -N content were observed in the first month after digestate application (Figures 1C,D). Ammonium contents between 150 and 200 mg N kg\(^{-1}\) were found at NW, which were double that measured at HF (80 mg N kg\(^{-1}\)) in this period. Following the initial peaks, a general decrease in soil \( \text{NH}_3^+ \) -N content was observed with time, with a faster rate of decrease at HF. Soil \( \text{NH}_3^+ \) -N contents were greatest for AD+NI and AD. A similar trend occurred for soil \( \text{NO}_3^- \) -N content (Figures 1E,F); however, the greatest \( \text{NO}_3^- \) -N concentrations were observed for treatments without the nitrification inhibitor (D and AD), within the first month following digestate application. Peak soil \( \text{NO}_3^- \) -N contents for D+NI and AD+NI were more constant through the whole experiment and their values were comparable with other treatments in the last 2 months at both sites. Soil \( \text{NH}_3^+ \) -N and \( \text{NO}_3^- \) -N contents were below 20 mg N kg\(^{-1}\) for the controls at both sites throughout the experiment (Figures 1C–F).

Nitrogen Losses
The percentage of total N applied lost as \( \text{NH}_3 \) and N\(_2\)O averaged across all “digestate treatments” were significantly higher at HF (17.4 and 0.45% of the total N applied, respectively) than at NW (11.6 and 0.13% of the total N applied, respectively; Table 4). The majority of the \( \text{NH}_3 \) loss occurred during the first and second days following digestate application (Figure 2). Cumulative \( \text{NH}_3 \) volatilization losses were significantly reduced by the acidification of the digestate (\( P < 0.001 \)), being 1.5% of the total N applied for the mean of AD and AD+NI treatments and 27.6% of the total N applied for the mean of D and D+NI treatments across both sites. Mean N\(_2\)O loss from digestate treatments with the nitrification inhibitor (D+NI and AD+NI) was 0.17 kg N ha\(^{-1}\) and 0.35 kg N ha\(^{-1}\) for those without the nitrification inhibitor, a >50% reduction although the differences were not significant (\( P = 0.097 \), Table 4). The peaks in daily N\(_2\)O emissions (Figure S3) were related to higher WFPS (Figure S1), especially for the “digestate” treatments at HF. The airline to one of the automatic chambers used to determine N\(_2\)O fluxes at HF appeared to be blocked (Figure S4, chamber 2 for AD treatment), so its values were replaced by the mean value of the other two chambers from the same treatment for statistical analysis because only three chambers per treatment were used.
Yield, Nitrogen Offtake, Nitrogen Use Efficiency (NUE), and Inorganic Nitrogen Replacement

Grain yield and total crop production were influenced by the site in a different way for the control and digestate treatments (Table 4). Higher mean grain yields ($P = 0.004$) were measured at NW ($8.93 \pm 0.37 \text{ t ha}^{-1}$) than at HF ($7.55 \pm 0.44 \text{ t ha}^{-1}$). The same effect was observed for plant production ($P < 0.001$), $15.36 \pm 0.45 \text{ t ha}^{-1}$ at NW and $11.37 \pm 0.50 \text{ t ha}^{-1}$ at HF. The application of the different digestate treatments resulted in a significant increase in grain yield ($P < 0.001$) and total crop production ($P < 0.001$) in relation to the control treatment (grain yield, $5.47 \pm 0.64 \text{ t ha}^{-1}$, and plant production, $11.09 \pm 1.36 \text{ t ha}^{-1}$) without N application but no significant differences were observed between the “digestate” treatments (grain yield,
Acidified Digestate Reduces N Losses

The properties of the digestate used in our field experiments were comparable to those reported elsewhere (Möller and Müller, 2012; Nkoa, 2014): high pH (≥7.0), low DM content, high proportion of total N as NH$_4^-$-N, negligible NO$_3^-$-N content, and similar total N, P, and K contents. In general, the application of digestate does not alter soil properties in the short-term but can increase microbial activity and biomass (Melero et al., 2016), N mineralization and NH$_3$ oxidation (Odlare et al., 2008), soil mineral N content (Möller et al., 2008), hydraulic conductivity, between 8.31 ± 0.47 t ha$^{-1}$ for D+NI and 9.52 ± 0.49 t ha$^{-1}$ for AD, and plant production, between 16.21 ± 0.66 t ha$^{-1}$ for D+NI and 16.95 ± 1.06 t ha$^{-1}$ for AD+NI; Table 4). The interaction site × treatment was not significant for grain yield because an analogous trend for the “digestate” treatments was observed at both sites (Figure 3), however, it was significant for plant production ($P = 0.024$), because the highest mean values were observed for AD > AD+NI > D > D+NI > C at HF, and for AD+NI > D+NI > D > AD > C at NW (Figure 4). Thousand grain weight was lower ($P < 0.001$) at NW (43.9 ± 0.5 g) than at HF (47.8 ± 0.4 g) and was reduced ($P < 0.001$) in the following order in relation to the different “digestate” treatments, C >, D+NI >, D >, AD > AD+NI (Table 4).

The mean grain N offtake and crop N offtake the means across the ‘digestate’ treatments were significantly higher at NW than at HF, but $NUE_g$ and $NUE_c$ were lower than at HF (Table 4). Digestate application significantly increased grain N offtake and crop N offtake in comparison to the control, as expected (Table 4). Highest N offtake values were from AD followed by AD+NI for both grain and crop, and $NUE_g$ and $NUE_c$ were also highest for these treatments although differences were not significant (Table 4). There were no significant site × treatment interactions for N offtake, $NUE_g$ or $NUE_c$.

Fertilizer replacement value was significantly greatest for AD and significantly least for D+NI for both grain and total crop yield at HF (Table S3) and followed the same order whether fitting a linear (or quadratic function; $P < 0.100$ for data calculated with both fittings): AD (168 ± 20 kg N ha$^{-1}$) > AD+NI (154 ± 18 kg N ha$^{-1}$) > D (137 ± 9 kg N ha$^{-1}$) > D+NI (111.1 ± 7.7 kg N ha$^{-1}$), for the linear approach and grain N fertilizer replacement. The differences between the linear and the quadratic approaches for the calculation of the inorganic N replacement by digestate were 4.5% for grain yield and 8.1% for total crop production. However, when fertilizer replacement was calculated as a function of the total N applied per treatment, the differences were not significant between the “digestate treatments” (Table S3) and ranged between 84.2 ± 5.9% for D+NI treatment and 103.6 ± 6.9% for D treatment. At NW, the fertilizer N response plots were severely affected by lodging and data were subsequently not used.

**DISCUSSION**

**Digestate and Soil Characteristics**

The properties of the digestate used in our field experiments were comparable to those reported elsewhere (Möller and Müller, 2012; Nkoa, 2014): high pH (≥7.0), low DM content, high proportion of total N as NH$_4^-$-N, negligible NO$_3^-$-N content, and similar total N, P, and K contents. In general, the application of digestate does not alter soil properties in the short-term but can increase microbial activity and biomass (Melero et al., 2016), N mineralization and NH$_3$ oxidation (Odlare et al., 2008), soil mineral N content (Möller et al., 2008), hydraulic conductivity,
and decrease soil bulk density (Garg et al., 2005), in relation to undigested feedstocks.

Unfortunately, the N loading rates for the different treatments in our study were not the same, and not exactly the 190 kg N ha\(^{-1}\) (equivalent) that we targeted. This is something that has been reported in other studies (Pezzolla et al., 2012; Riva et al., 2016). To address this, the N losses were presented as a percentage of the total N applied. The variability of the feedstocks, digestate handling, transport, and storage in the local biogas plants and in tanks in the fields before the application could have caused changes in the digestate N content between the initial sampling time and the time of land application. It is well known that open stores (Wang et al., 2014), and the lack of semi-permeable materials to cover the tanks (Börjesson and Berglund, 2007) and protective gas-tight layers (Battini et al., 2014) can lead to large N losses, predominantly via NH\(_3\) volatilization (Petersen and Sørensen, 2008; Fangueiro et al., 2015a), in comparison with the undigested feedstocks. Moreover, the pH of our digestate was 8.24 ± 0.01 at HF and 8.05 ± 0.01 at NW, and according to Muck and Steenhuis (1982), very high losses of NH\(_3\) from digestate occurs above pH 8.0, and small losses below pH 6.0. The lower pH of the acidified digestate applied to our fields (5.40 ± 0.01 at HF and 2.88 ± 0.06) and the time between acidification and field application of the digestate (1 week at HF and 2 days at NW) would also contribute in part to explain the higher N content in relation to the non-acidified digestate because the equilibrium NH\(_4^+\)/NH\(_3\) favors volatilization at higher pH (Möller, 2015). The tanks used for the storage of the digestate in our fields before application had a simple thread lid and were only loosely fixed to prevent pressurization of the tanks, so were not gas-tight, which may have contributed to greater N loss via NH\(_3\) volatilization, especially from the non-acidified treatment (Möller, 2015).

The different dry matter (DM) content (%) of the digestate applied at HF (3.08 ± 0.19 for D and 5.08 ± 0.04 for AD) and at NW (7.52 ± 0.08 for D and 9.66 ± 0.03 for AD) explains the variable distribution of the applied digestate at both sites following simulated bandspreading. The higher DM content at NW resulted in discrete bands of digestate, whereas the lower DM content at HF meant that the digestate did not remain within bands resulting in a more homogeneous distribution covering almost the whole surface of the plots that received digestate. The higher surface area of digestate in contact with the air at HF helps to explain the higher N losses at HF (mainly as NH\(_3\) but also as N\(_2\)O despite the lower WFPS at HF, Figure S1 and Table 4), and differences in soil NH\(_4^+\)-N and NO\(_3^-\)-N contents between NW and HF (Figure 1), especially during the first months after digestate application. In this study, the higher DM content for NW digestate compared with that at HF did not result in higher NH\(_3\) emissions as would be expected for slurries (e.g., Misselbrook et al., 2004), suggesting that other factors (such as the increased emitting surface area) were important in controlling NH\(_3\) volatilization. The greater post-harvest soil NO\(_3^-\)-N content at NW could indicate more risk of leaching than at HF (Figures 1E,F).

### Nitrogen Losses: Acidification and Nitrification Inhibitors

Acidification to a pH of <6.0 reduced NH\(_3\) volatilization to <2.0% of the total N applied (AD and AD+NI plots), a similar reduction to that reported by other authors when non-acidified digestate or slurries were injected into the soil (Fangueiro et al., 2015b; Riva et al., 2016; Baral et al., 2017). These values were significantly lower than when the digestate was not acidified (D and D+NI), resulting in NH\(_3\) losses of more than 27% of the total applied N (Table 4). High NH\(_4^+\) content and pH of the digestate facilitate N losses via NH\(_3\) volatilization (Fangueiro et al., 2015a; Möller, 2015) that can account up to more than a 40% of the total applied N if not managed carefully (e.g., Riva et al., 2016; Nicholson et al., 2017). Our results for the digestate treatments when the digestate was not acidified (D and D+NI) are consistent with these studies. Ammonia is quickly emitted, normally during the first few hours after slurry (Ni et al., 2012) or digestate (Figure 2 of this experiment; Nicholson et al., 2017) are applied. Consequently, measures to reduce its emission should be focused in the first few hours after application (e.g., rapid incorporation) and on production or storage phases of the digestate, to reduce N losses at the different phases. The large, significant decrease in N losses from NH\(_3\) volatilization we measured following acidification of digestate (ca. 95% reduction compared with non-acidified digestate) demonstrates the effectiveness of this method to control and reduce these emissions, addressing a key knowledge gap identified by Nicholson et al. (2017).

Although more experiments under different weather conditions, physico-chemical soil properties and crops are necessary, our study supports the use of acidification of food based digestate, consistent with this technique being called the Best Available Technology (BAT) for reducing NH\(_3\) losses from slurries in some countries (Kai et al., 2008). Rapid soil incorporation has also been shown to reduce NH\(_3\) losses by up to a 85% when following application of food waste based digestate (Tiwary et al., 2015) but it could increase N losses in the form of N\(_2\)O as observed for slurries (Thorman, 2011).

When the pH of the digestate is >6.00 the high soil NH\(_4^+\) contents after the application of the digestate stimulate nitrification (Muck and Steenhuis, 1982), and, consequently, N\(_2\)O emissions. The intensive frequency of N\(_2\)O sampling and analysis at HF (Figure S3), and the higher mineralizable N measured at HF (Table 1) might explain the greater cumulative N\(_2\)O losses compared to NW, as some N\(_2\)O peaks may have been missed because of the lower frequency of sampling at NW. Nitrification could have been responsible for most of the N\(_2\)O emissions because the WFPS was always <50% at both sites (between ≈10 and 25% at HF and between 15 and 50% at NW, Figures S1A,B) and the N\(_2\)O peaks were related to higher WFPS in soil (Figures S1, S3; Zhu et al., 2013).

Nitrous oxide emissions as a result of denitrification are stimulated after the application of organic amendments with a large content of C (Rochette et al., 2006). Therefore, we do not discard that denitrification was, in part, responsible of some N\(_2\)O emissions observed after digestate application (Figure S3), although the initial NO\(_3^-\)-N contents in soil were
FIGURE 2 | Time course of ammonia volatilization (means ± standard error) during the week after digestate application at Henfaes (HF, A) and North Wyke (NW, B). D, digestate; D+NI, digestate plus nitrification inhibitors; AD, acidified digestate; AD+NI, acidified digestate plus nitrification inhibitors; n = 4 for each treatment.

FIGURE 3 | Grain yields (means ± standard error) at Henfaes (HF, A) and North Wyke (NW, B) at the end of the experiment as a function of the treatment. C, control; D, digestate; D+NI, digestate plus nitrification inhibitors; AD, acidified digestate; AD+NI, acidified digestate plus nitrification inhibitors; and several rates of N applied as NH$_4$NO$_3$, including the control treatment (C, 0 kg N ha$^{-1}$); n = 5 for each treatment. Different letters indicate differences according to Tukey’s HSD test at a probability level of 0.05.
FIGURE 4 | Plant production (straw and grain, means ± standard error) at Henfaes (HF, A) and North Wyke (NW, B) at the end of the experiment as a function of the treatment. D, digestate; D+NI, digestate plus nitrification inhibitors; AD, acidified digestate; AD+NI, acidified digestate plus nitrification inhibitors; and several rates of N applied as NH$_4$NO$_3$, including the control treatment (C, 0 kg N ha$^{-1}$); n = 5 for each treatment. Different letters indicate differences according to Tukey’s HSD test at a probability level of 0.05.

lower (Figures 1E,F) than in a previous study by Fangueiro et al. (2015b) where high soil NO$_3^-$-N content (c. 80 mg kg$^{-1}$) resulted in significant N$_2$O emissions. In addition, hot spots where both nitrification and denitrification processes occur are created in soil after the addition of organic manures, including even when bulk WFPS is below 50%, resulting in N$_2$O emissions (MarkFoged et al., 2011; Zhu et al., 2015). Baral et al. (2017) found that the highest N$_2$O emissions were produced at WFPS between 53 and 56% in a field experiment in which spring barley was fertilized with manure and digestate and that coupled nitrification-denitrification was the source of these emissions.

A decrease in the nitrification process was observed for the treatments in which DMPP was added; i.e. higher NH$_4^+$-N and lower NO$_3^-$-N contents were measured at both experimental sites for D+NI and AD+NI treatments during the experiment (Figures 1C–F). The addition of DMPP resulted in a reduction of N$_2$O emission of up to a 50% in comparison to the digestate without the nitrification inhibitor (D and AD), although the differences were not significant ($P = 0.097$, Table 4). The use of nitrification inhibitors such as DMPP and dicyandiamide (DCD) have been proved to be an effective strategy to reduce N losses from soils where mineral fertilizers (Liu et al., 2013) or slurries (Vallejo et al., 2005; Fangueiro et al., 2016) are applied. The acidification of slurries has also been shown to delay nitrification in some soils (Fangueiro et al., 2013) but not in others, e.g., soils with a high buffering capacity where the soil pH was not altered after the application of the acidified digestate (Fangueiro et al., 2016). Owusu-Twum et al. (2017) recently demonstrated in a short-term experiment under controlled conditions that acidification of slurries could significantly reduce N$_2$O emissions, but to a lesser extent than when DMPP was used. We found some evidence of a delay in the nitrification process for the acidified digestate, where peak soil NO$_3^-$-N content was observed a few weeks later than for unacidified digestate at HF (Figure 1E), and soil NH$_4^+$-N contents were higher for AD than for D on the majority of measurement occasions (Figures 1C,D), although this could also be attributed to the initial higher NH$_4^+$-N contents of the acidified digestate (Table 3). This inhibition of nitrification could have been caused by the decrease in soil pH after spreading the acidified digestate, an effect that was persistent until the
end of the experiment, because the population and activity of denitrifying bacteria is affected by soil pH (Gandhapudi et al., 2006). However, acidification did not alter N₂O emissions (these were only affected by the addition of DMPP). The presence of a substantial amount of C and inorganic N could have promoted completed denitrification to N₂ for AD and AD+NI treatments (where the nitrifying bacteria activity could have been inhibited by acidification) as indicated by Pezzolla et al. (2012) with comparable WPFS values for soils amended with digestate. The percentage of applied N lost via N₂O in our experiment ranged between 0.13 and 0.45% (Table 4), in accordance with 0.10–0.41% calculated by Baral et al. (2017) and with 0.45 ± 0.15% reported by Nicholson et al. (2017) under comparable conditions, all lower than the 1% default IPCC emission factor (IPCC, 2006).

Nitrogen Uptake, Nitrogen Use Efficiency, Fertilizer Replacement Rates, and Yields

Although grain and crop N offtakes were improved when the applied digestate was acidified, the differences were not significant for NUEᵣ or NUEₑ (Table 4). The results for HF indicate that digestate can be an effective replacement for inorganic fertilizers such as NH₄NO₃ in terms of crop production (Figures 3, 4). These results are in agreement with similar experiments: Walsh et al. (2012) for a grassland, Riva et al. (2016) for a maize silage crop in which they used manure- and crop-based digestates, Furukawa and Hasegawa (2006) for spinach, Haraldsen et al. (2011) for barley, and Pezzolla et al. (2012) for a grassland using food waste based digestate. On the one hand, yields for D and D+NI treatments were similar to those obtained for doses of inorganic N of 136.7 ± 9.1 and 111.1 ± 7.7 kg N ha⁻¹, and the mean values were higher when the digestate was acidified, i.e., AD and AD+NI, which produced similar yields to doses of 168.3 ± 20 and 154.2 ± 17.5 kg N ha⁻¹ at HF. However, no significant differences were found between the different “digestate treatments” (D, D+NI, AD, and AD+NI) when these fertilizer replacement values are based on the total N applied with each “digestate treatment” at HF. The reduction of yields observed in our experiment (only for the mean values, not significantly) when NI were added to the digestate in comparison to the treatments without NI agrees with Misselbrook et al. (2014) but not with the increase in yields reported by Abalos et al. (2014) in their meta analysis. However, in order to achieve effective mitigation of N losses and fertilizer replacement values, digestate should be acidified or rapidly incorporated into the soil following application, as shown in this experiment and by Möller et al. (2008), respectively.

CONCLUSIONS

Acidification of digestate and the inclusion of a nitrification inhibitor are good strategies for the utilization of food waste based digestates because they contributed to the mitigation of N losses following application to a winter wheat crop. Without acidification, NH₃ volatilization accounted for almost a 30% of the total N applied in digestate. This emission was reduced by 95% with acidification. We demonstrated that wheat yields when acidified digestate was applied at HF (176 kg N ha⁻¹) were similar to these produced by an inorganic N form (NH₄NO₃) applied at a rate of 154–168 kg N ha⁻¹. Acidification of the digestate seems to be an effective technique after digestate spreading, producing higher mean yields and inorganic N replacements than when the digestate is not acidified. Without the acidification of the digestate, NH₃ volatilization accounted for almost a 30% of the total N applied resulting in a serious economic cost and environmental damage. This study encourages the use of digestate from the anaerobic digestion of food waste alongside acidification and with the addition of a nitrification inhibitor, as an environmentally sound option for N management. However, the reduction in soil pH that was measured in the acidified treatments at both sites, suggest that the effect of slurry and digestate acidification on soil quality and function needs to be assessed in the long-term.

AUTHOR CONTRIBUTIONS

AS-R, AC, RS, DC, DJ, and TM: Conceptualization; AS-R, AC, JH, KS, and JC: Formal analysis; DC, DJ, and TM: Funding acquisition; AS-R, and AC: Investigation; AS-R, AC, RS, JH, KS, and JC: Methodology; DC, DJ, and TM: Supervision; AS-R, Wrote the manuscript; All the authors review and approved the last version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2018.00035/full#supplementary-material
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