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Contrasting effects of inhibitors and biostimulants on agronomic performance and reactive nitrogen losses during irrigated potato production

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ABSTRACT

Urea is the dominant form of nitrogen (N) fertilizer used globally. Various additives have been designed for co-application with urea to improve performance of N-intensive crops including potato (*Solanum tuberosum* L.). Few if any studies have compared ‘inhibitor’ additives with ‘biostimulants’ designed to enhance plant growth or microbial activity. Over two potato growing seasons (2015–2016) in an irrigated loamy sand in Minnesota, we quantified agronomic performance and N losses as both nitrate (NO_3^-) and nitrous oxide (N_2O) in treatments receiving urea, with and without additives including: nitrification inhibitors dicyandiamide (DCD) or 3,4-dimethylpyrazole phosphate (DMPP), alone or combined with the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT), or a biostimulant containing N-fixing microbes (NFM) by itself or combined with an amino acid blend (AAB). The biostimulants produced modest (~10%) improvements in tuber yield, under limited conditions, compared to urea alone. However, NFM increased N_2O emissions by 32–56%, in contrast to the inhibitors, which decreased N_2O emissions by 42–75%. Compared to urea alone, the inhibitors tended to increase soil ammonium and decrease soil NO_3^- concentrations; however, no differences in soil inorganic N in the upper 0.3 m of the profile were observed with the biostimulants. During the growing season with greater rates of soil water flux (2015), none of the inhibitors decreased NO_3^- leaching, while NFM increased NO_3^- leaching by 23%. When AAB was combined with NFM, reactive N losses did not differ from the urea-only treatment. Biostimulants can have unintended impacts on reactive N losses and should be used with caution pending additional study to better understand their effects on biological processes, and to quantify their performance in other agro-ecosystems.

1. Introduction

Potato is a major global food crop, with several countries in Asia, North and South America, Europe and Africa producing greater than 1 million metric tons (Mt) per year. In 2016, the top five leading nations for potato production were China (100 Mt), India (44 Mt), Russian Federation (31 Mt), Ukraine (22 Mt) and USA (20 Mt) (FAO, 2018a). Due to its inherent physiological requirements, and because potatoes are frequently grown in coarse-textured soils, relatively large rates of N fertilizer are often applied. For example, recommended N rates (RNR) for potatoes in the northern USA range from 180 to 390 kg N ha^{-1} y^{-1} for yield goals above 50 Mg tuber ha^{-1} (Lang et al., 1999; Rosen, 2018). Thus, potato production has high potential for reactive N losses, which can occur in soluble form as NO_3- with potential impacts on local and regional water quality (Padilla et al., 2018), and/or in gaseous forms including N_2O, which impacts stratospheric ozone depletion (Ravishankara et al., 2009) and global atmospheric radiative forcing (Tian et al., 2016). A number of modeling and empirical studies have been conducted across the globe to simulate or measure NO_3- or N_2O losses in potato systems under conventional or alternative management regimes (Burton et al., 2008; Hyatt et al., 2010; Venterea et al., 2011; Kim et al., 2015; Zareabyaneh and Bayatvarkeshi, 2015; Woli et al., 2016).

Urea is the predominant chemical form of N fertilizer used globally, accounting for approximately 74% of total agricultural N fertilizer used worldwide in 2016, including 67% as urea and 7% in urea-containing solutions (FAO, 2018b). Various ‘additives’ have been promoted for co-application with urea to improve agronomic performance of N-intensive crops, while also decreasing reactive N losses. Many of these products are designed to inhibit one or more of the soil biochemical processes that promote reactive N losses, primarily focused on either nitrification or urea hydrolysis. Various formulations and combinations of these products have been evaluated to reduce NO_3^- leaching and/or N_2O emissions and/or ammonia (NH_3) volatilization losses. The large

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Table 1
Selected soil chemical properties.[†]

Year	[‡]pH (water)	O.M %	NO_3^--N	P	K mg kg^{-1}	Ca	Mg	SO_4	Zn	Cu	Fe	Mn	B
2015	6.2	2.2	4.5	40.1	126	855	152	2.8	1.8	0.7	34.5	8.7	0.3
2016	6.1	1.6	2.7	32.3	121	787	126	1.0	1.1	0.5	27.3	10.4	0.2

† Samples were collected before spring planting from the 0 to 0.15-m depth, except for inorganic NO_3^--N samples, which were collected from the 0 to 0.6-m depth.
‡ Methods: Soil pH (1:1, soil/water), organic matter (loss on ignition), NO_3^--N (KCl extractable) P (Bray-P1), K, Ca, Mg (ammonium acetate extractable), B (hot water), SO_4^{2-}-S, and Zn, Cu, Fe, Mn (DTPA).

number of published site-level studies in this space has allowed for meta-analyses of their effects, with contrasting conclusions (Quemada et al., 2013; Pan et al., 2016; Thapa et al., 2016; Eagle et al., 2017; Lam et al., 2017; Cantarella et al., 2018; Li et al., 2018). Studies have also documented the limitations of available inhibitors, which include susceptibility to decomposition, leaching or deactivation once applied to soil, depending on temperature, moisture, pH and other factors (Kelliher et al., 2008; Engel et al., 2015; McGeough et al., 2016; Barrena et al., 2017; Guardia et al., 2018) and inconsistent agronomic benefits sufficient to justify their cost (Yang et al., 2016; Rose et al., 2018). These limitations have spurred efforts to develop more effective products, and to compare performance of different products. Two currently available nitrification inhibitors, DCD and DMPP, target the same microbial enzyme system (i.e., autotrophic NH_3 oxidation mediated by nitrifying bacteria) but have different chemical characteristics, which may affect their relative persistence and performance (Friedl et al., 2017). While there have been some site-level comparisons of DCD and DMPP on crop yields and/or greenhouse gas emissions (Liu et al., 2013; Soares et al., 2015; Weiske et al., 2001), no studies have conducted a comprehensive comparative assessment of their agronomic performance concurrently with effects on NO_3- leaching and N_2O emissions.

In addition to inhibitors, other products, often referred to as 'biostimulants', have been promoted to increase productivity of agronomic and horticultural plants (du Jardin, 2015). Within the category of biostimulants are products containing microbial organisms and/or different organic compounds with varying proposed modes of action (du Jardin, 2012; Calvo et al., 2014; Halpern et al., 2015). Specific N-fixing bacteria, including *Azotobacter* and *Clostridium* sp., have been evaluated for their potential to increase N availability in the plant-soil system (Kennedy et al., 2004). Chitin-derived compounds have been found favorable for promoting beneficial soil microbial growth (Sharp, 2013). Studies have also assessed the performance of different crops, such as cucumber, tomato, green bean, potato, soybean, and wheat to amino acids application (Souri et al., 2017; Popko et al., 2018; Röder et al., 2018; Teixeira et al., 2018). However, only limited reports of the performance of such biostimulants products are available in peer-reviewed literature (Abbas et al., 2014; Katiyar et al., 2015; Rodrigues et al., 2018). Therefore, we hypothesized that the use of nitrification and urease inhibitors or N-fixing microorganisms is a potential strategy to improve soil N availability to meet potato crop N demand and hence reduce fertilizer-induced N losses.

The objective of this study was to conduct a comprehensive field assessment of the agronomic and environmental impacts of different inhibitor and biostimulant additives when co-applied with urea for irrigated potato production in the upper Midwest USA. We compared the performance of DCD and DMPP, alone or combined with NBPT, and a biostimulant containing N-fixing microbes (NFM), alone or combined with a biostimulant amino acid blend (AAB), on potato yield and quality, NO_3^- and N_2O losses and soil N availability during the 2015 and 2016 growing seasons in an irrigated loamy sand in Minnesota. We also evaluated crop, NO_3^- leaching and soil N responses to varying N rates in the absence of additives (i.e., with urea only). The simultaneous quantification of NO_3^- leaching and chamber-based measurements of direct N_2O emissions allowed us to also estimate indirect N_2O emissions resulting from N_2O produced from off-site transformation of leached NO_3^- to N_2O using published emission factors as done in previous studies (Venterea et al., 2011; Maharjan et al., 2014).

2. Material and methods

2.1. Site description, experimental design and production practices

The experiment was conducted during the 2015 and 2016 growing seasons on two immediately adjacent (within 20 m) field sites at the Sand Plain Research Farm in Becker, MN (45°23′ N, 93°53′ W). Soil at the site is a Hubbard loamy sand (sandy, mixed, frigid Entic Hapludolls) containing 82% sand, 10% silt and 8% clay. Representative soil samples were collected from the upper 0.15 m (or upper 0.6 m for extractable NO_3^-) before implementation of experiments each spring. Results for cations and anions are reported in Table 1. The 30-yr (1981–2010) average precipitation during Apr through Oct is 610 mm (Minnesota DNR, 2019). An onsite weather station recorded air temperature and precipitation at 10-min intervals.

Both fields (60 × 40 m) were planted with non-irrigated and unfertilized rye (*Secale cereale* L.) for two years preceding each experimental growing season. Rye was harvested in summer followed by a rye cover crop that was incorporated the following spring before planting. One week prior to planting, KCl and $K_2SO_4{\cdot}2MgSO_4$ were broadcast and incorporated with a chisel plow at 178 kg K, 25 kg Mg and 41 kg S per ha to partially satisfy the K, S and Mg recommendations for irrigated potato in Minnesota (Rosen, 2018). At planting each year, in all treatments, starter fertilizer was banded 80 mm to the side and 50 mm below the seed piece using a metered, drop-fed applicator. Starter consisted of diammonium phosphate (DAP), KCl, $K_2SO_4{\cdot}2MgSO_4$, and $ZnSO_4$, providing 34 kg N, 37 kg P, 150 kg K, 22 kg Mg, 47 kg S, 1.1 kg B and 1.1 kg Zn per ha.

Plots were laid out in a randomized complete block design with four replicates of fourteen different N management treatments, with the same treatments evaluated in both years. Each plot consisted of seven 6.0-m long rows, with two central rows used for vine and tuber harvest. Russet Burbank, the most widely grown potato cultivar used for processing in the upper Midwest USA, was planted. Whole "B" seed potatoes were hand planted on 30 Apr 2015 and 22 Apr 2016 with 0.9- and 0.3-m spacing between and within rows, respectively. Emergence occurred on 26 May 2015 and 23 May 2016. Plant response variables were measured in all four replications, while soil and environmental response variables were measured in three replications of each treatment.

In addition to the 34 kg N ha^{-1} of DAP at planting, urea-N was applied in two equal sidedressings, which were manually applied on the soil surface next to the potato rows and incorporated by mechanical hilling on the same day. The first N sidedress/hilling events occurred on 21 May 2015 and 01 Jun 2016, and the second on 02 June 2015 and 09 June 2016. Timing of the first N sidedress in 2016 was delayed due to prohibitively wet soil conditions during the optimum application period. Two separate experiments were conducted in parallel: (i) an 'N rate study' which evaluated the effect of N application rate in the absence of additives, and (ii) an 'additive study' which compare the effects of the different additives. The N rate study consisted of four treatments receiving DAP and urea, with no additives, at total N rates of 34 (DAP only), 168, 252 and 336 kg N ha^{-1}, corresponding to 13, 67, 100 and 133% of the recommended N rate (RNR), respectively, based on best management practices for this system (Rosen, 2018). The additive study included two of the above treatments (urea at 67 and 100% of RNR with no additives) together with 10 additional treatments which included five different additives, or additive combinations, each co-applied with urea at 67 and 100% of the RNR, including: (i) DCD applied at 5% of the total N rate (equivalent to 8.4-12.6 kg ha^{-1}), (ii) DMPP applied at 0.5% of the total N rate (0.84-1.26 kg ha^{-1}) (Benckiser et al., 2013; Chen et al., 2014; Zerulla et al., 2001), (iii) DMPP, at 0.5% of the N rate, combined with NBPT applied at 0.13% of the N rate ($0.22-0.33$ kg ha^{-1}), consistent with the commercially available NBPT-urea product (UTEC®, Eurochem Agro), (iv) a commercial inoculant mixture referred to here as 'NFM' (HYTa®, Agrinos) which contained the N-fixing microbes *Azotobacter vinelandii* and *Clostrium pasteurianum* as well other 'secondary organisms' including *Pseudomonas fluorescens* and *Nitrosomonas*, *Nitrococcus, Nitrobacter* and *Bacillus* spp., and (v) NFM combined with a commercial amino acid blend referred to here as 'AAB' (HYTb®, Agrinos) containing 17 different *L*-amino acids comprising 3% of the mixture as well as 'trace' amounts of chitin, chitosan, and glucosamine. The NFM and AAB products were provided by Eurochem Agro in liquid form and ready to use. Based on their marketing literature and proposed modes of action, NFM and AAB are referred to here as 'biostimulants' while DCD, DMPP and NBPT are referred to as 'inhibitors'. The inhibitors were pre-mixed with urea prills in the laboratory at the desired rates and allowed to air dry to generate a coated urea for application. The biostimulants were spray-applied on the soil surface using 2.5 L ha^{-1} of liquid product diluted into 200 L water ha^{-1} and according to manufacturer recommendations. Treatments receiving NFM alone received one surface-spray application at planting and a second application within one day of the second side-dress N application each year. Treatments receiving NFM + AAB received one 2.5 L ha^{-1} application of both products at planting and two additional applications of AAB only, one at potato inflorescence (02 Jul 2015 and 24 Jun 2016) and another about two weeks later (17 Jul 2015 and 08 Jul 2016).

Irrigation was applied uniformly across all treatments through a solid-set overhead sprinkler system and scheduled by the checkbook method, which estimates daily evapotranspiration (ET) and soil water deficit (Wright, 2002). Irrigation water was periodically sampled and analyzed for NO_3^- using the diffusion–conductivity method (Carlson et al., 1990). Irrigation water contained 8–11 mg NO_3^- -N L^{-1}, which contributed 20 and 22 kg N ha^{-1} in 2015 and 2016, respectively. Consistent with Maharjan et al. (2016), irrigation N inputs were not included in the data analysis because farmers typically do not consider these inputs in their N management planning, unless NO_3^- -N concentrations are consistently above 10 mg L^{-1} (Lamb et al., 2015), and because irrigation can increase leaching losses of N, thus negating the increased N input (Maharjan et al., 2014).

2.2. Nitrous oxide emissions

Soil-to-atmosphere N_2O fluxes were measured using stainless steel non-steady state chambers (Venterea et al., 2005) in 8 of the 16 treatments: the treatment receiving only DAP (34 kg N ha^{-1}) and the treatments receiving urea at 100% of RNR, with and without additives. In 2015, fluxes were measured on 31 dates between 29 Apr and 2 Oct, and in 2016 fluxes were measured on 36 dates between 21 Apr and 7 Oct. Each year, fluxes were measured once before planting in 24 locations within the experimental area. Following planting, two stainless steel chamber anchors (0.50 by 0.29 by 0.09 m deep) were installed in each plot, one between potato rows and one adjacent to the potato plants, on the hill of potato rows. Fluxes were then measured twice weekly from the first N sidedress application through July, and once weekly thereafter. On each sampling day, between 900 and 1200 h local time (Cosentino et al., 2012, 2013), insulated and vented chamber tops (0.50 by 0.29 by 0.10 m high) were attached to anchors with binder clips, and gas samples were collected using 12-mL polypropylene syringes at 0, 0.5, 1, and 1.5 h after sealing the chambers. Samples were immediately transferred to glass vials sealed with butyl rubber septa and analyzed within two weeks using a headspace auto sampler (Teledyne Tekmar) connected to a gas chromatograph (Model 5890, Agilent/Hewlett-Packard) equipped with an electron capture detector (Venterea et al., 2005) which was calibrated daily using analytical-grade standards (Scott Specialty Gases). Fluxes of N_2O were calculated from the rate of change in chamber N_2O concentration using the restricted quadratic regression procedure (Parkin et al., 2012) and the chamber bias correction method to account for the suppression of the surface-atmosphere concentration gradient (Venterea, 2010). Values obtained from the hill and inter-row chamber locations were averaged to obtain a single daily flux value for each plot, since each location represented approximately equal areas within each plot (Hyatt et al., 2010). Trapezoidal integration of daily fluxes was used to calculate cumulative growing season area-scaled N_2O emissions (Parkin and Venterea, 2010). Yield-scaled N_2O emissions were calculated as the ratio of cumulative area-scaled N_2O emissions to potato tuber yield. The emissions factor (EF) for direct N_2O emissions was calculated by subtracting the cumulative N_2O observed in each urea-amended plot from cumulative N_2O in the corresponding starter-only plot within each block, divided by the urea N application rate. Indirect N_2O emissions were determined by multiplying cumulative growing season NO_3-leaching (described below) by the indirect N_2O emission factor (EF_5) of 0.75% (De Klein et al., 2006). Indirect N_2O emissions were then added to direct N_2O emissions to estimate total direct + indirect N_2O emissions (Venterea et al., 2011; Maharjan et al., 2014).

2.3. Soil inorganic N concentrations

Soil samples for determining NH_4^+ and NO_3^- concentrations were collected from the 0-0.3 m depth every two weeks. A composite sample was generated for each plot, consisting of five soil cores distributed evenly across the hill and furrow. For determination of residual soil NO_3^-, samples were taken after tuber harvest from the 0-0.6 m depth. Soil samples were extracted with 2 *M* KCl at a soil:solution ratio of 1:5 (*w/v*), and inorganic N concentrations were determined using the diffusion–conductivity method (Carlson et al., 1990). Time-integrated (TI-) NH_4^+ and NO_3^- concentrations were calculated by trapezoidal integration of concentration versus time to provide a measure of cumulative soil N availability as used in previous studies (Burton et al., 2008; Maharjan and Venterea, 2013).

2.4. Soil water nitrate concentrations and leaching

During the growing season, water samples from below the rooting zone were collected approximately once per week using suction cup lysimeters (Venterea et al., 2011) installed at the 1.2-m depth in three out of the four blocks for each treatment. Each lysimeter consisted of a round-bottom, 100-kPa high-flow porous ceramic cup (60 mm long by 48 mm o.d. and 44 mm i.d.) (Soilmoisture Equipment Corp.) affixed with epoxy to the end of a 1.3-m long by 48-mm i.d. polyvinylchloride (PVC) pipe. On the opposing end of the PVC pipe, two polyethylene tubes (5.35 mm i.d.) were inserted through a rubber stopper, with the 'vent' tube extending into the pipe to 0.1 m below the stopper and the other 'sample' tube extending to 2 mm above the ceramic cup. Both vent and sample tubes were connected to 6 mm i.d. plastic (Tygon) tubing equipped with polypropylene ratcheting clamps. Water samples were collected by applying 40 kPa vacuum to the sample tube and transferred to 50-mL polypropylene vials, capped and stored frozen until analysis for NO_3^- using the diffusion–conductivity method (Carlson et al., 1990).

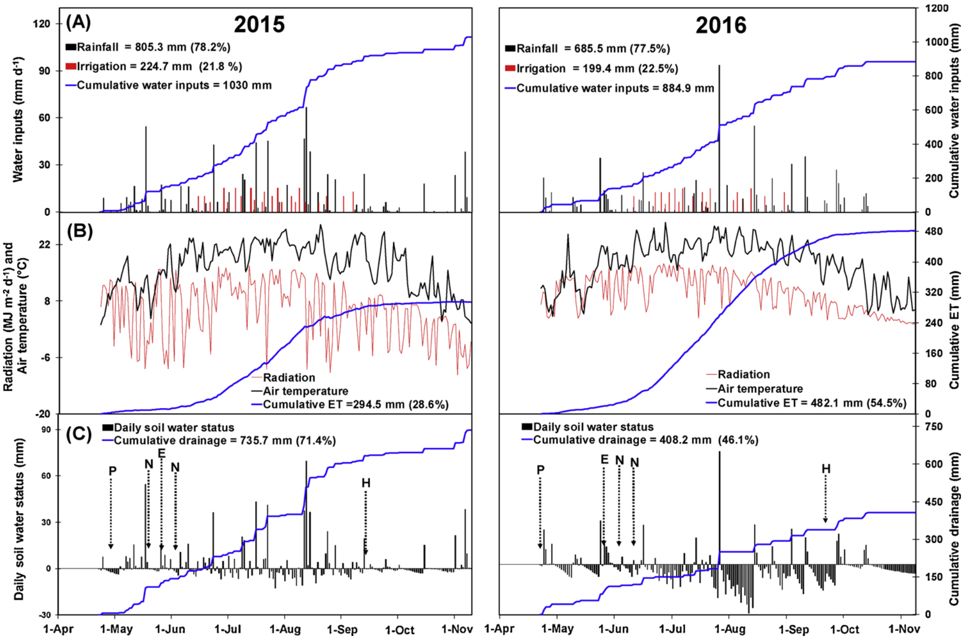

Fig. 1. (A) Daily and cumulative water inputs, (B) daily radiation, air temperature and cumulative evapotranspiration, and (C) daily soil water status and cumulative drainage in 2015 and 2016. Daily and cumulative variables are displayed on left and right axes, respectively. In (C), daily water status value > 0 indicate leaching volumes and values < 0 indicate drying below field capacity. Arrows indicate planting (P), emergence (E), sidedressing (N) and harvest (H) dates. Percentages (%) of total water inputs are shown in parentheses.

The volume of water drained below the root zone was estimated using the approach of several previous studies conducted at this same research site (Errebhi et al., 1998; Venterea et al., 2011; Maharjan et al., 2014) using the following water balance equation

$$D = (P + I) - (\mathrm{ET} + \Delta S) \tag{1}$$

where D is daily water drainage volume, P is precipitation, I is irrigation water applied, ET is evapotranspiration, and ΔS is the daily change in soil profile water storage (S), with all terms having units of mm d^{-1}. An on-site National Weather Service catch-can and gauge stick system was used for determining P and I. Daily ET (mm d^{-1}) was calculated using the Penman–Monteith equation (Allen et al., 1998; Venterea et al., 2011) for a grass reference crop:

$$\mathrm{ET} = \frac{0.408\Delta(R_n - G) + \gamma\frac{900}{T+273}u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \tag{2}$$

where R_n is net radiation at the crop surface (MJ m^{-2} d^{-1}), G is the net soil heat flux density (MJ m^{-2} d^{-1}), which was assumed to be zero for daily calculations, T is the air temperature (°C), u_2 is the wind speed (m s^{-1}), e_s and e_a are the saturation and actual vapor pressures (kPa), respectively, Δ (kPa°C^{-1}) is the slope of the relationship between e_s and T, and γ is the psychrometric constant (0.065 kPa°C^{-1}). The ET values obtained from Eq. [2] were adjusted for potato crop stage using a crop coefficient given by Stegman et al. (1977) and Allen et al. (1998).

Potential daily drainage (*PDD*) was determined by adding the difference between daily water inputs and outputs, i.e., $(P + I) - ET$, to the current value of S. When *PDD* exceeded available water holding capacity (*AWC*) in the top 1.2 m of soil profile, then D was calculated from *PDD* – *AWC*. The *AWC* to 1.2 m was assumed to be 140 mm based on previous measurements at this site (Gremy et al., 1993) confirmed by Maharjan et al. (2014). Daily NO_3^- leaching was then determined as the product of D and same-day NO_3^- concentrations, with the latter quantity determined using linear interpolation of observed lysimeter water NO_3^- concentrations for days on which NO_3^- concentrations were not measured. Growing season NO_3^- leaching was calculated as the sum of the daily NO_3^- leaching amounts.

2.5. Tuber yield and crop N uptake

Vines from two 6.0-m sections of rows were manually harvested on 03 Sep 2015 and 13 Sep 2016 and weighed within a week before mechanical tuber harvest, which occurred on 8 Sep 2015 and 20 Sep 2016. Tubers were sorted into weight classes to determine total yield and proportion of tubers > 170 g ($T_{>170}$) as an indication of tuber quality. Vine and tuber sub-samples from each plot were collected to determine dry matter and N content. Samples were dried at 60 °C, weighed for dry matter yield, and then ground with a Wiley mill to pass a 2-mm sieve. Total N in ground samples was determined using a combustion analyzer (Elementar Vario EL, Laurel, NJ) using standard procedures (Horneck and Miller, 1998). Crop N uptake was calculated as the products of tissue N concentration and dry matter yield of vines and tubers. The cumulative growing degree days (GDD) was determined as a sum of GDD [(average of maximum and minimum air temperatures) - 2 °C] (Oliveira et al., 2016) from crop emergence to vine harvest.

2.6. Data analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, 2010) with N rate and year (for the N rate study), or N rate, year, and additive (for the additive study) treated as fixed effects, and block and interactions with block considered as random effects. Based on inspection of residuals, none of the data were transformed prior to analysis. Means were compared using independent pairwise t tests at $P \le 0.05$ using the PDIFF option in PROC MIXED (SAS Institute, 2010). In the N rate analysis, orthogonal contrasts were used to determine the nature of the N rate effect and its interaction with year. Relationships among variables were evaluated with correlation and regression modules in Excel (Microsoft) and Statistix (Analytical Software, Tallahassee, FL).

3. Results

3.1. Climate and soil water

Seasonal precipitation (1 Apr through 31 Oct) was 32% and 12% above the 30-yr average (610 mm) in 2015 and 2016, respectively (Fig. 1A). The difference in precipitation between growing seasons was more pronounced during the periods following the largest inputs of N fertilizer. For example, after the first N sidedress through the end of June, precipitation was 55% greater in 2015 (133 mm) than in 2016 (86 mm). There was also less cumulative ET in 2015, due to lower daily

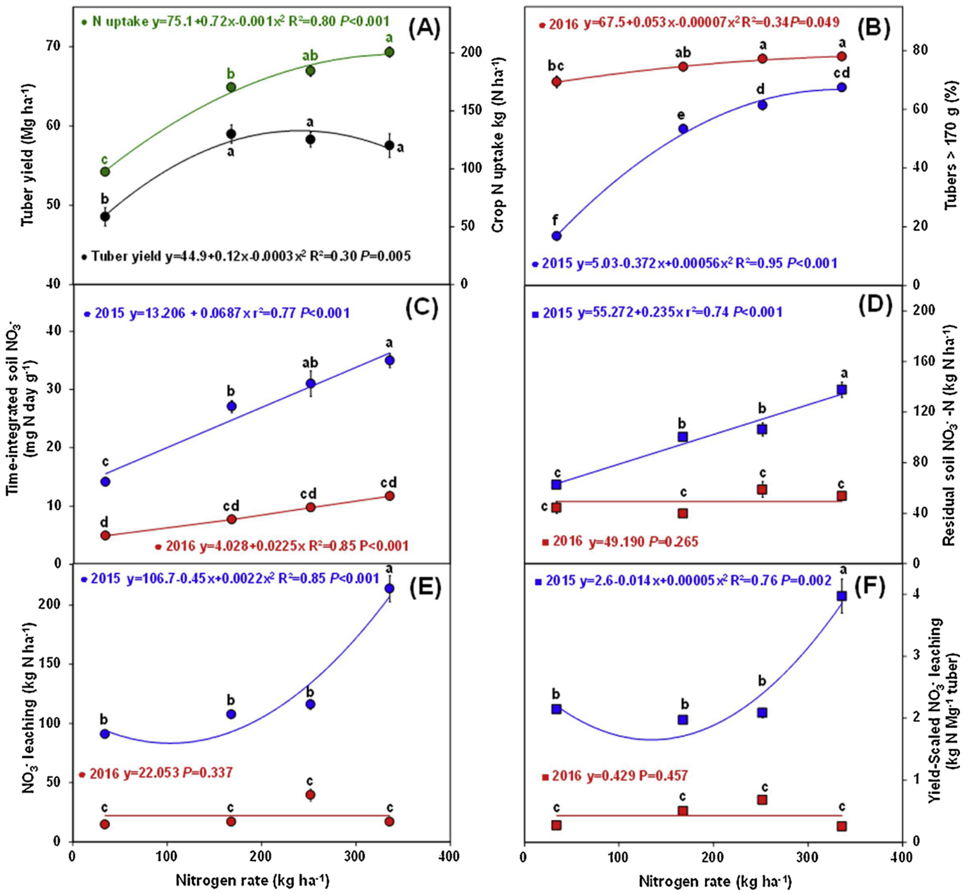

Fig. 2. Variation by N rate in (A) tuber yield (left axis) and crop N uptake (right axis) averaged across both years, and (B) proportion of tubers > 170 g, (C) time-integrated soil nitrate, (D) residual soil N and (E) area- and (F) yield-scaled nitrate leaching, shown separately for 2015 and 2016. Within each variable, means accompanied by the same letter are not significantly different at $P < 0.05$.

average air temperature (16.4 °C) and radiation (5.6 MJ m^{-2} d^{-1}) compared to 17.2 °C and 10.0 MJ m^{-2} d^{-1}, respectively, in 2016 (Fig. 1B). These climatic differences resulted in 80% greater cumulative water drainage in 2015 than 2016 (Fig. 1C), and in 1825 and 2153 cumulative GDD, respectively.

3.2. Agronomic responses

In the N rate study, tuber yields, crop N uptake and $T_{>170}$ were 18, 11 and 30% greater in 2016 than 2015, respectively, when averaged across N rates, and all three variables displayed significant second-order polynomial responses to N rate (Fig. 2A-B). Mean yields did not differ significantly among treatments receiving ≥ 67% of RNR, while mean crop N uptake was 18% greater at 133% compared to 67% of RNR (Table 2). There was a significant year-by-N rate interaction effect on $T_{>170}$ resulting from a larger increase in $T_{>170}$ with N rate in 2015 than in 2016 (Fig. 2B).

In the additive study, there was a significant year-by-additive-by-N rate interaction effect on tuber yield, with differences among treatments ranging from 7 to 13% (Table 3, Fig. 3A). In 2015, at 67% of RNR, the DCD and NFM + AAB treatments had 10% greater tuber yield than the urea-only treatment. In 2016, at 67% of RNR, the DCD treatment had 7–12% lower yield than all other treatments, while at 100% of RNR, DCD had 8% greater yield than the urea-only treatment, and the NFM treatment had 9–13% greater yield than all other treatments except DCD. There was a significant year-by-N rate interaction effect on $T_{>170}$. In 2015, averaged across additive treatments, $T_{>170}$ was significantly greater at 100% (61.8%) than at 67% of RNR (55.3%), and these mean values were 26% and 20% lower, respectively, than mean $T_{>170}$ at each corresponding N rate in 2016. The N rates did not influence $T_{>170}$ in 2016. There was a significant year-by-additive interaction effect on crop N uptake (Table 3 and Fig. 3C). In 2015, DMPP had 12% greater crop N uptake than the urea-only treatment. In 2016, NFM had 16% greater mean crop N uptake than NFM + AAB. Crop N uptake was greater in 2016 than 2015 in the urea-only and NFM treatments.

3.3. Soil inorganic N concentrations

Mean soil NH_4^+ concentrations ranged from 0.05–35 mg N kg^{-1} in 2015 and from 0.05–25 mg N kg^{-1}in 2016 (Fig. S1). Mean soil NO_3- concentrations ranged from 1 to 40 mg N kg^{-1} in 2015 and 1–15 mg N kg^{-1}in 2016 (Fig. S2). In the N rate study, TI-NH_4^+ was greater in all urea-amended treatments compared to the starter-only treatment and was 84% greater at 133% compared to 67% of RNR (Table 2). There was a significant year-by-N rate interaction effect on TI-NO_3- and residual soil NO_3–N, with significant linear relationships observed between N rate and TI-NO_3- in both years, and between N rate and residual soil NO_3–N in 2015 (Fig. 2C-D).

In the additive study, there was a significant year-by-additive-by-N rate interaction effect on TI-NH_4^+ (Table 3, Fig. 3B). The overall trend was for inhibitor treatments to have greater TI-NH_4^+ than the urea-only treatment. In 2015, this effect was significant for the DCD, DMPP and DMPP + NBPT treatments at 67% RNR, and for the DCD and DMPP treatments at 100% RNR, relative to the urea-only treatment. The DCD treatment also had greater TI-NH_4^+ than several other additive treatments at both 67 and 100% of RNR, as did the DMPP treatment at 100% of RNR. In contrast, in 2016, only the DMPP + NBPT treatment at 100% of RNR had significantly greater TI-NH_4^+ than the urea-only treatment as well as greater TI-NH_4^+ than all other treatments except DMPP alone.

In contrast with TI-NH_4^+, TI-NO_3^- was significantly less with the inhibitors compared to the urea-only treatment across both N rates and years (Table 3). However, for both biostimulants treatments (NFM and

Table 2
Means of response variables and significance of F values for fixed sources of variation in the N rate study.

Source of variation	Tuber yield Mg ha^{-1}	Tubers > 170 g %	Crop N uptake kg N ha^{-1}	NO_3^- leaching	Yield-scaled NO_3^- leaching kg N Mg^{-1} tuber	[δ]TI-NH_4^+ mg N day g^{-1}	TI-NO_3^-	Residual soil N kg N ha^{-1}
Year								
2015	51.1b[¶]	46.7	154b	132	2.54	9.76	26.7	102
2016	60.4a	74.7	172a	22.0	0.37	5.17	8.47	49.2
Significance	**	**	*	**	**	ns[†]	**	**
N rate								
kg N ha^{-1} (% RNR)[¥]								
34 (13)	48.5b	43.0	97.2c	52.9	1.20	2.88c	9.50	53.5
168 (67)	58.9a	63.8	170b	62.2	1.12	6.26b	17.3	70.0
252 (100)	58.2a	69.2	184ab	77.8	1.38	9.32b	20.3	82.5
336 (133)	57.5a	72.6	201a	115.5	2.12	11.4a	23.2	95.7
Significance	*	**	**	**	**	**	**	**
Interaction[‡]								
Year × N rate	ns	**	ns	**	**	ns	*	*

[†] ns = not significant at $P \leq 0.05$.
[¶] Within a column, means followed by same lowercase letter are not significantly different at P ≤ 0.05. Letters are not shown for significant main effects that also had significant interaction effects.
[‡] Means comparisons for variables having significant interaction effects are shown in Fig. 2.
[δ] Time-integrated (TI) soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations.
[¥] Percent of recommended N rate.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

NFM + AAB), TI-NO_3^- did not differ from the urea-only treatment, and both treatments had greater TI-NO_3^- than the DCD treatment. Residual soil NO_3^--N was nearly twice as large in 2015 than 2016 (Table 3).

3.4. Nitrate leaching

Lysimeter NO_3^- concentrations ranged from approximately 2–60 mg N L^{-1} in 2015 and from 0.1–25 mg N L^{-1} in 2016 (Fig. S3). In the N rate study, there was a significant year-by-N rate interaction effect on area- and yield-scaled NO_3^- leaching (Table 2). Greater NO_3^- leaching was observed in 2015 than 2016 at all N rates, and significant second-order polynomial relationships were observed between N rate and NO_3^- leaching and in 2015 but not in 2016 (Fig. 3E–F).

In the additive study, there was a significant year-by-additive interaction effect on area- and yield-scaled NO_3^- leaching (Table 3, Fig. 3D–E). The amount of NO_3^- leached in 2015 was consistently greater than in 2016 by a factor of 4–8 for each N rate-additive combination. None of the additive treatments resulted in a decrease in NO_3^- leaching, at either N rate or in either year, compared to urea-only. In 2015, the NFM treatment had 21 and 23% greater area- and

Table 3
Means of response variables and significance of F values for fixed sources of variation in the additive study.

Source of variation	Tuber yield Mg ha^{-1}	Tubers > 170 g %	Crop N uptake kg N ha^{-1}	NO_3^- leaching	Yield-scaled NO_3^- leaching kg N Mg^{-1} tuber	[δ]TI-NH_4^+ mg N day g^{-1}	TI-NO_3^- kg N ha^{-1}	Residual soil N
Year								
2015	56.9	58.6	180	120	2.10	15.7	26.3a[¶]	96.0a
2016	63.7	75.8	188	21.9	0.35	7.14	7.40b	49.6b
Significance	*	**	[†]ns	**	**	*	**	**
Additive								
None (urea only)	58.6	66.5	177	70.0	1.25	7.79	18.8a	76.2
DCD	60.2	66.7	189	66.3	1.12	15.8	14.0d	69.8
DMPP	60.8	67.1	187	72.7	1.24	13.7	15.8cd	74.1
DMPP + NBPT	60.5	66.5	185	71.4	1.20	12.8	16.2bcd	72.6
NFM	61.3	67.8	191	79.2	1.38	9.32	17.5abc	76.8
NFM + AAB	60.2	68.5	176	64.7	1.13	9.01	18.4ab	67.0
Significance	ns	ns	ns	ns	ns	**	**	ns
N rate								
67 % of RNR	60.5	65.0	175b	67.0	1.11	10.2	15.5	69.1
100 % of RNR	60.1	69.4	194a	74.4	1.24	12.6	17.8	76.5
Significance	ns	*	**	ns	ns	ns	ns	ns
Interaction[‡]								
Year × Additive	ns	ns	*	*	*	**	ns	ns
Year × N rate	ns	*	ns	ns	ns	ns	ns	ns
Additive × N rate	ns	ns	ns	ns	ns	ns	ns	ns
Year × Additive × N rate	*	ns	ns	ns	ns	*	ns	ns

[†] ns = not significant at $P \leq 0.05$.
[¶] Within a column, means followed by same lowercase letter are not significantly different at P ≤ 0.05. Letters are not shown for significant main effects that also had significant interaction effects.
[‡] Means comparisons for variables having significant interaction effects are shown in Fig. 3 or discussed in text.
[δ] Time-integrated (TI) soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

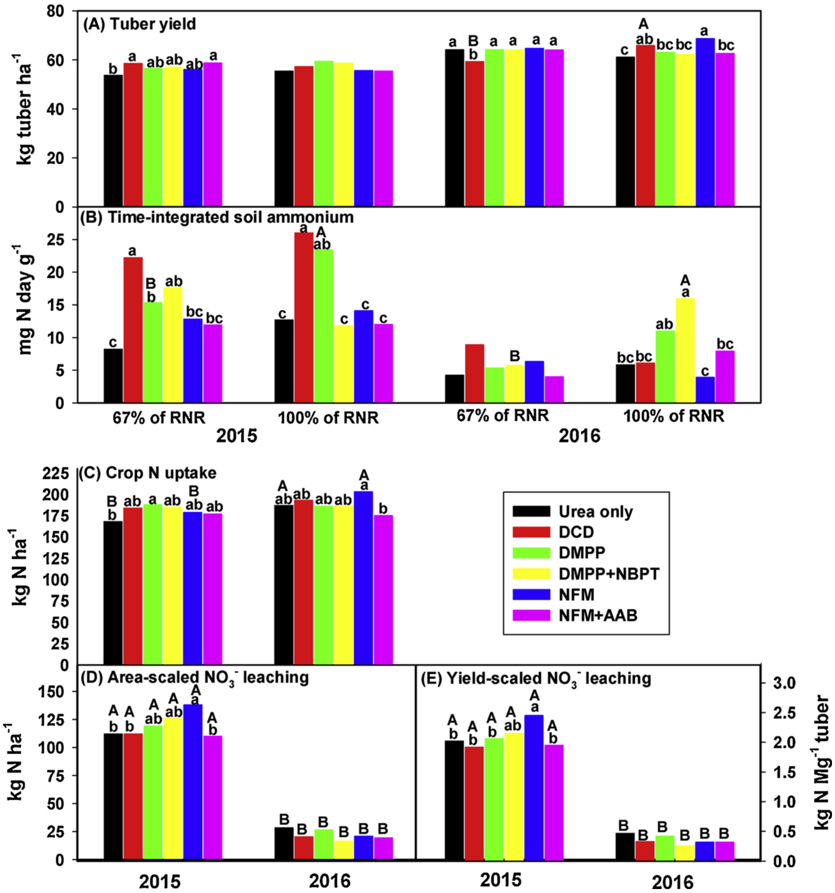

Fig. 3. Mean response variables in the additive study. Within each year and N rate (for A and B), or within each year (for C through E), bars with same lower case letter are not significantly different by additive. In A and B, upper case letters indicate significant differences by N rate within each year and additive. In C through E, upper case letters indicate significant differences by year within each additive. All means comparisons made at $P < 0.05$.

yield-scaled NO_3^- leaching than the urea-only treatment, respectively. No differences by additive treatment were observed in 2016. Across the N rate and additive study and both seasons, NO_3^- leaching was positively correlated with TI-NH_4^+ ($r^2 = 0.40$) and TI-NO_3^- ($r^2 = 0.78$) ($P < 0.001$). Within each season, these relationships were not as strong, and were only significant in 2015 when NO_3^- leaching was positively correlated with TI-NH_4^+ ($r^2 = 0.11$) and TI-NO_3^- ($r^2 = 0.10$) ($P < 0.05$).

3.5. Nitrous oxide emissions

Soil N_2O fluxes measured in the treatments receiving 100% of RNR, and in the starter-only treatment, ranged from < 5–200 µg N $m^2\,h^{-1}$ in 2015 and from < 5–140 µg N $m^2\,h^{-1}$ in 2016 (Fig. 4). All three inhibitor treatments had significantly lower cumulative area- and yield-scaled N_2O emissions, EF, and total direct + indirect N_2O emissions, than the urea-only treatment, with no significant differences among inhibitor treatments (Table 4). On average, inhibitor treatments reduced these variables by 54, 58, 75 and 42%, respectively, compared to the urea-only treatment. In contrast, the NFM treatment had 39% greater area-scaled N_2O emissions, a 56% greater EF, and 32% greater total direct + indirect N_2O emissions compared to the urea-only treatment. For yield-scaled N_2O emissions, the pairwise comparison between the NFM and urea-only treatments yielded a P-value of 0.06. The NFM + AAB treatment did not differ from the urea-only treatment on any of these variables. Cumulative N_2O emissions were positively correlated with TI-NO_3- in 2015 ($r^2 = 0.41$) and 2016 ($r^2 = 0.42$), and with NO_3- leaching in 2015 ($r^2 = 0.20$) and 2016 ($r^2 = 0.31$), but not with TI-NH_4^+. When evaluated across both seasons, none of these relationships were significant.

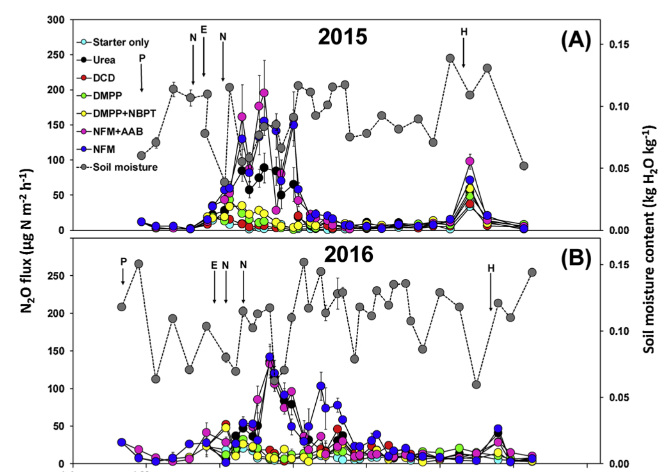

Fig. 4. Daily mean (and standard error) N_2O flux (left axes) and soil moisture content (right axes) in treatments receiving urea with or without additives at 100% of recommended N rate (RNR) and the starter-only control that received 13% of RNR. Arrows indicate planting (P), emergence (E), N sidedressing (N) and harvest (H) dates.

4. Discussion

4.1. Agronomic performance

Growing season had a dominant effect on tuber yield and tuber size in this study. Total yields were about 15% higher in 2016 compared to 2015. The greater yields in 2016 were presumably due to higher radiation and warmer temperatures in 2016 relative to 2015 (Oliveira et al., 2016). While tuber yield response to N was similar between the

Table 4
Mean N_2O emissions and significance of F values for fixed sources of variation in treatments receiving urea with or without additives at 100% of recommended N rate.

Source of variation	Direct N_2O emissions			Direct + Indirect N_2O emissions
	Area-scaled	Yield-scaled	Emissions factor	Area-scaled
	kg N ha^{-1}	g N Mg^{-1} tuber	%	kg N ha^{-1}
Year				
2015	1.31	23.6	0.41	2.10
2016	1.30	20.9	0.36	1.38
Significance	ns[†]	ns	ns	ns
Additive				
Starter-only[‡]	0.47	9.7	–	0.87
None (Urea only)	1.65b[¥]	29.5a	0.54b	2.23b
DCD	0.69c	11.4b	0.10c	1.19c
DMPP	0.79c	13.1b	0.15c	1.37c
DMPP + NBPT	0.78c	12.9b	0.15c	1.33c
NFM	2.30a	38.7a	0.84a	2.95a
NFM + AAB	1.83ab	32.0a	0.63ab	2.35ab
Significance	**	**	***	**
Year × Additive	ns	ns	ns	ns

† ns = not significant at $P \leq 0.05$.
‡ Data from the starter-only control were used to determine emissions factor (EF) but were not used in the analysis of additive effects because it received only 13% of recommended N rate.
¥ Within a column, means followed by same lowercase letter are not significantly different at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

two years, $T_{>170}$ increased more dramatically with increasing N rate in 2015 than in 2016 even though overall tuber size was greater in 2016 than in 2015. These results suggest that the growing conditions in 2016 were more conducive for tuber bulking regardless of the amount of N supplied. Reasons for a greater N response in 2015 are not clear, but may have been affected by complex interactions among environmental cues such as temperature, light, and nutrition (Ewing, 1997). The proportion of larger-sized tubers in processing potatoes such as Russet Burbank is a key attribute to provide net monetary returns for growers (Zebarth and Rosen, 2007; Wilson et al., 2009).

Although NFM and AAB coated urea were found to increase yield compared with urea alone over the two-year study, the effects were not consistent and depended on year and N rate. For example, we observed increases in tuber yield when NFM and AAB were co-applied with urea in 2015 at 67% RNR; however, in 2016 only the use of NFM at the RNR increased yield. Biostimulants had no effect on tuber size, nor did they significantly affect N uptake. Yield responses to NFM and AAB reported in previous studies are also variable. For example, Brown et al. (1964) reported no yield response to inoculation with *Azotobacter*, while Imam and Badawy (1978) reported a positive effect in one of three potato cultivars tested. The large 15–30% tuber yield increases reported by Falcón-Rodríguez et al. (2017) with chitosan application could not be substantiated in the present study. Aïzi and Cheba (2015) demonstrated that soil application of chitin and chitosan eliminates phytopathogenic fungi leading to an increase in N fixing bacteria. However, overall response to chitosan appears to be highly influenced by environmental conditions making it difficult to predict consistent benefits (Hadwiger, 2013). More systematic approaches are needed to identify conditions under which responses to these additives occur.

In this study, the only microbial inhibitor affecting yield was DCD, and the effect was somewhat inconsistent. Relative to urea alone, DCD increased tuber yield in 2015 at 67% RNR but yields were lower at 67% RNR and higher at the RNR in 2016. Inhibitors had no effect on tuber size, but DMPP alone increased N uptake relative to urea alone in 2015, suggesting an increase in N availability under some conditions with DMPP. Kelling et al. (2011) found that DCD decreased marketable tuber yields, which was attributed to negative effects of ammonium nutrition on potato growth. In previous research, DCD and DMPP with ammonium nitrate fertilizer sources increased potato yields suggesting a more suitable balance of inorganic N forms in the soil during potato cultivation in those studies (Kelling et al., 2011; Souza et al., 2019). In the present study, soil ammonium was higher with DCD in 2015 relative to urea alone at both N rates, but only at 67% RNR in 2016 (Figure S1). Ammonium nutrition may have played a role in the negative response at 67% RNR in 2016, but it is unclear why this effect was not observed in 2015 and requires further study.

4.2. Reactive N losses

The nitrification inhibitors examined here (DCD and DMPP) have been shown to reduce N_2O emissions in a variety of cropping systems (Bell et al., 2015; Migliorati et al., 2014; Soares et al., 2016; Tian et al., 2015; Yang et al., 2016). A potential advantage of DMPP over DCD is that lower addition rates are required for effective reduction, which could reduce both cost and unintended effects, such as the potential for DCD to be absorbed by crops and animals (Torralbo et al., 2017). Here, with regard to N_2O mitigation, we found that DMPP applied at 0.5% of the N rate performed similarly to DCD applied at 5% of the N rate (equivalent to a DMPP:DCD ratio of 1:10). Using ammonium sulfate nitrate as the N fertilizer and a DMPP:DCD ratio of 1:10, Weiske et al (2001) found that DMPP was nearly twice as effective as DCD for reducing N_2O in a clayey loam with total N rates ranging from 90 to 180 kg N ha^{-1}. However, with urea as the N source, and over a range of DCD application rates (1.0–5.0% of the N rate) and DMPP:DCD ratios (1:2 to 1:5), DCD and DMPP have performed similarly to each other in reducing N_2O emissions (Di and Cameron, 2012; Liu et al., 2013; Soares et al., 2015).

While at least one study has compared DMPP with the urease inhibitor NBPT for mitigating N_2O emissions (Lam et al., 2018), as far as we know, this is the first study to compare DMPP alone with DMPP + NBPT; here we found no difference in the effects of a single versus dual inhibitors on N_2O reduction. In cases where different inhibitors have similar effects on N_2O, selection of the 'best' inhibitor should ideally be based on a comprehensive evaluation of their relative costs, agronomic performance and impacts on other environmental variables. Further studies are needed to better define application rates that optimize these factors, and to determine how inhibitor performance may vary with site-specific conditions. For example, a recent laboratory study indicated that temperature can affect the relative performance of DMPP and DCD for reducing N_2O production (Lan et al., 2018).

Both DCD and DMPP have been shown to be effective in reducing NO_3^- leaching (e.g., Di and Cameron, 2012). While all inhibitor treatments in the current study reduced cumulative soil NO_3^- concentrations in the upper 0.3 m, none of them reduced NO_3^- leaching compared to urea alone. In the N rate study, NO_3^- leaching was relatively unresponsive to N rate, except at 133% of RNR in 2015. This suggests that a substantial fraction of the NO_3^- leached in treatments receiving ≤ 100% of RNR did not originate from N fertilizer, but from mineralization of soil organic matter. Relatively high rates of NO_3^- leaching (15–68 kg N ha^{-1} y^{-1}) have been observed in this soil (Zvomuya et al., 2003), and other coarse-textured soils in the region (29–73 kg N ha^{-1} y^{-1}) (Struffert et al., 2016), in plots not receiving N fertilizer inputs. In spite of their coarse-texture, the loamy sands in this region of Minnesota tend to have organic matter content in surface soil that ranges from 1.5 to above 4%, which was enhanced by the rye crop that was incorporated prior to planting each year in the current study. Since the inhibitors were co-applied with urea and then concentrated to some extent in the hills, the inhibitors would not likely affect nitrification of any mineralized organic N occurring outside of the applied region or throughout the soil profile, which could have contributed substantially to NO_3^- leaching. In contrast, the greater effectiveness of the inhibitors for reducing N_2O suggests that the N_2O was derived mainly from the applied urea. This interpretation was supported by paired *t*-tests indicating significantly greater ($P < 0.001$) N_2O fluxes from chambers placed in the row position compared to chambers placed between rows, as shown previously in similarly managed potato crops at this site (Hyatt et al., 2010). Urea transformation results in specific chemical and biochemical effects, including alteration of soil pH and microbial NH_3 toxicity, which can promote N_2O production, while these effects would not be expected to occur during mineralization of soil organic matter (Venterea et al., 2015). The large inter-annual difference observed in NO_3^- leaching, which was approximately six times greater in 2015 than 2016, was due to a combination of 80% more water flowing through the soil profile and greater NO_3^- concentrations in 2015. The field used in 2015 had 38% greater soil organic matter (0 to 0.15 m) and 67% greater soil NO_3^- levels (0 to 0.6 m), both of which likely contributed to the large inter-annual differences. Similarly, large year-to-year differences in NO_3^- concentrations have been reported in previous studies on this same soil type (Zvomuya et al., 2003).

The most unexpected result of the current study was the > 30% increase in N_2O emissions with addition of the NFM biostimulant product compared to urea alone, which was also accompanied by a > 20% increase in NO_3^- leaching in one growing season (2015). The latter result (increased NO_3^- leaching) indicates an increase in soil N availability with NFM, which could have contributed to the former result (increased N_2O). However, beyond the consistency of these two findings, the underlying mechanisms responsible for these effects will require further study. To our knowledge, unintended negative environmental impacts of microbe-based biostimulant products have not been previously reported under field conditions. In a laboratory study, Calvo et al. (2013) found that a sandy loam-sand mixture amended with urea and treated with a bacterial inoculant containing *Bacillus*, *Acidovorax* and *Rhodococcus* spp. resulted in 40% greater N_2O production compared to urea alone; however, in treatments amended with urea-ammonium nitrate (UAN) instead of urea, N_2O emissions were substantially decreased when the inoculant was added. Calvo et al. (2013) suggested that the decreased N_2O observed with UAN could be explained by microbial metabolites, including phenolic compounds, which may inhibit nitrification (Chaabouni et al., 2012). The explanation proposed by Calvo et al. (2013) for the elevated N_2O emissions in the urea + inoculant treatment was that NH_3 resulting from urea hydrolysis could have been toxic to the added microbes, inactivating any beneficial responses and also releasing C and N that could promote nitrification and/or denitrification. The increase in N_2O emissions and NO_3^- leaching observed with NFM in the current study suggests that the added microbes influenced one or more soil processes affecting soil N availability and/or mobility, e.g. N fixation, mineralization, nitrification. Similar to Calvo et al. (2013), we did not find elevated inorganic N in the upper 0.30 m to support this, but we did find elevated NO_3^- in water samples from below the root zone (1.2 m). The lack of differences in soil N observed in the upper part of the soil profile may have resulted from rapid leaching in this coarse-textured soil. Some evidence for increased soil N supply was also provided by agronomic data; i.e., in 2016, crop N uptake across both N rates and tuber yield at 100% of RNR were greater with NFM than urea-alone. Thus, N-fixation may have indeed been enhanced by the free-living microbes (*Azotobacter vinelandii* and *Clostrium pasteurianum*) present in NFM. In addition, NFM also contained nitrifying bacteria of the genera *Nitrosomonas*, *Nitrococcus* and *Nitrobacter* which could have increased rates of NH_4^+ oxidation (i.e., nitrification) that can promote N_2O production either directly during nitrification itself, or indirectly via increased production of NO_3^- which can then be denitrified to N_2O (Butterbach-Bahl et al., 2013). Also, changes in the populations or activities of ammonia-oxidizing nitrifier populations (such as *Nitrosomonas* spp.) relative to that of nitrite-oxidizing populations (such as *Nitrobacter* spp.) can result in decoupling of the two process which can also stimulate nitrification-driven N_2O production (Venterea et al., 2015; Breuillin-Sessoms et al., 2017). Further study, including controlled laboratory studies, are needed to better understand the relative importance of changes in nitrifying activity and N-fixing activity following additions of biostimulant products containing both classes of microbes.

The effects of NFM on reactive N losses were largely counteracted when the AAB product was applied together with NFM. The amino acids contained in the AAB product including glycine, phenylanine and glutamine have been shown to increase crop nitrate content and productivity in laboratory, greenhouse and field experiments (Souri et al., 2017; Röder et al., 2018; Teixeira et al., 2018; Popko et al., 2018). Here, we found no evidence that AAB addition increased total above-ground crop N uptake or yield, but it did appear to prevent increases in N_2O emissions and NO_3^- leaching that occurred with NFM in the absence of AAB. The other constituents of the AAB product, including chitin, have also been evaluated for their ability to improve plant nutrition through a variety of potential mechanisms including enhancing the activity of chitinolytic microbes (Sharp, 2013). Again, the exact mechanisms responsible for the effects observed here cannot be clearly deduced based on this field study, indicating that additional study is needed to better understand biostimulant effects on biological processes, both at the laboratory scale and in the field to quantify their performance in other agro-ecosystems.

5. Conclusions

The inhibitors DCD and DMPP slowed nitrification as indicated by a trend of reduced soil NO_3^- and consistent mitigation of N_2O emissions. Although these inhibitors generally retained soil N as NH_4^+, they did not decrease NO_3^- leaching suggesting that a substantial fraction of the NO_3^- leached in this study likely originated from mineralization of soil organic matter (SOM) occurring outside of the zone of fertilizer and inhibitor application. Although N application increased the proportion of large tubers up to 100% of the recommended N rate, the additional N derived from mineralization resulted in sufficient available N for tuber yield when N fertilizer was applied at 67% of the recommended N rate for this site. Therefore, additives (inhibitors and biostimulants) had only modest agronomic benefits due to adequate N availability. In contrast to the inhibitors, a biostimulant containing N-fixing micro-organisms (NFM) when co-applied with urea actually increased N_2O emissions by 42–75% relative to urea alone across both years of the study; and, during the growing season with greater rates of soil water flux, NFM increased NO_3^- leaching by 23%. The combination of NFM and a second biostimulant comprised primarily of an amino acid blend (AAB) did not alter reactive N losses relative to urea alone. Overall, our results suggest that when potato is cultivated with urea to meet crop demands, nitrification inhibitors can mitigate N_2O emissions. However, biostimulants should be used with caution pending additional study to better understand their effects on biological processes and to quantify their performance in other agro-ecosystems.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fcr.2019.05.001.

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