Biosynthetically produced amino acid byproducts can replace nitrogen fertilizers for corn production

by

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DEDICATION

A special dedicatory to God for giving me all the marvelous people and things I have had and met in life. Not less important is the dedicatory to my family that have always supported me from a distance. This work goes with special dedication to my lovely daughter “Darielita” for being the light and the force that motivates me daily.

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AF I	Agronomy Research Farm site in 2014
AN	ammonium nitrate
AS	ammonium sulfate
HI	harvest index
IAA	indole-3-acetic acid
LAI	leaf area index
LYS	Lysine
NDVI	normalized difference vegetation index
NHI	nitrogen harvest index
PGRs	plant growth regulators
SF I	Sorenson Research Farm site in 2013
SF II	Sorenson Research Farm site in 2014
SPAD	leaf greenness
TDM	total dry matter
TRP	Tryptophan

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CHAPTER 1. LITERATURE REVIEW

The application of industrial byproducts to crop land has been the subject of extensive research. Various studies have reported the suitability of using some industrial byproducts like flue gas desulfurization gypsum obtained from coal combustion for energy production (DeSutter and Cihacek, 2009), food-processing byproducts such as soybean meal and vegetable pulp (Sims and Stehouwer, 2008), wood-processing byproducts like paper mill residuals (Sims and Stehouwer, 2008) and biotechnology byproducts such as amino acids sludge (Zhu et al., 1995; Martinez and Tabatabai, 1997) as either potential sources of nutrients for agricultural production or as soil amendments.

Amino acids such as tryptophan (TRP) and lysine (LYS) are two of the main products of the fermentation biotechnology industry (Deloitte, 2014). Both amino acids are considered essential in monogastric animals and human nutrition (Leuchtenberger et al., 2005; Huang et al., 2006) and they both are present in corn (*Zea mays* L.). However, most of the current cultivated corn grain is deficient in TRP and LYS (Huang et al., 2004; Scott et al., 2004) for monogastrics. These two feed amino acids are produced by fermentation processes using modified strains of bacteria (FAO, 2002) and are mainly added to feed as supplements to enhance and balance the nutritional quality of diets (Huang et al., 2006).

Feed amino acids such as TRP, LYS and threonine are the main biosynthetically produced amino acids and account for roughly 60% of the total amino acid market (Leuchtenberger et al., 2005). Furthermore, the global market for biosynthetically produced amino acids has steadily increased during the last decades and projections still trend upward. For instance, LYS and TRP global production increased about 3.5 and 14 fold, respectively, in the period between 2001 and 2013 (Ajinomoto Inc., 2004 and 2014). Along with the substantial increases in feed amino acid production the amount of byproducts produced was also raised and alternative uses are needed to avoid continued disposal of these byproducts into landfills or legally assigned sites. The chemical constituents and properties of feed amino acid byproducts make them attractive for agricultural uses, though positive and negative impacts may arise from their usage.

Benefits and drawbacks of land applied biosynthesis byproducts have been reported. Henning (2007) reported that LYS byproducts have potential to be used as a nitrogen (N) source for corn production in Iowa, USA. However, that same report acknowledged the potential risks associated with LYS byproducts utilization in terms of soil acidification. Other research studies conducted under greenhouse and laboratory conditions demonstrated the potential of different biotechnology byproducts including LYS byproduct to supply N to corn plants and their value as soil amendments (Zhu et al., 1995; Martinez and Tabatabai, 1997). The adoption of biotechnology byproducts for agricultural purposes will not only benefit corn farmers but also the bio-fermentation industry by creating new value-added products. In the USA, Iowa has a large feed amino acid industry, mainly because it has the largest amount of land devoted to corn grain production in the USA and the sugar derived from this cereal grain is one of the key raw materials for production of fermented amino acids (Ikeda, 2003).

In 2013, over 39 million hectares of corn were planted in the United States, and Iowa had the largest corn crop planted with over 5.7 million hectares (NASS, 2013). Corn producers are constantly looking for alternatives to reduce production costs. In this sense the growing production of feed amino acids and the concomitant increase in their byproduct production provides a potential means to corn producers to increase revenues by adopting the utilization of biosynthetically produced byproducts and reducing the associated costs with synthetic N fertilizer application. In 2010, the US corn crop received about 5 million metric tons of N fertilizer (ERS, 2011), which is roughly 45% of the total N fertilizer used in the US that year. In addition amino acids byproducts also contain organic carbon (C) and residual amino acids that may improve crop productivity by affecting soil processes or plant physiological processes.

Plant growth regulators (PGRs) are organic compounds that can impact plant growth at low concentration (Frankenberger and Arshad, 1995; Arshad and Frankenberger, 1998; Khalid et al., 2006). The PGRs play important roles in controlling plant growth and development. Among the five major types of PGRs, auxins are one of the most important for controlling plant growth and development. Plants respond to endogenously produced and exogenously applied auxins at various growth stages (Frankenberger and Arshad, 1995; Khalid et al., 2006). Auxins are plant hormones and indole-3-acetic acid (IAA) is the principal naturally occurring and the most physiologically active auxin in plants (Frankenberger and Arshad, 1995; Ahmad et al., 2007). A number of indole compounds and phenylacetic derivatives have been reported with auxin activity.

According to Bidwell (1979) the regulation of plant growth and reproduction could be achieved with different PGRs such as naturally occurring regulators including amino acids. Amino acids are organic nitrogenous compounds that serve as the building blocks for the synthesis of proteins in plants (Bidwell, 1979). In addition to its inclusion in peptide and protein syntheses, the amino acid TRP also is an important physiological precursor for biosynthesis of auxins in plants (Khalid et al., 2001; Qureshi et al., 2012). Metabolism of TRP results in the formation of many products including niacin (kynurenine pathway) and serotonin (hydroxylation) in addition to auxins (Martens and Frankenberger, 1993). Furthermore, the presence of auxins in soils may have ecological impacts influencing plant growth and development (Sarwar et al., 1993).

Soil applied TRP was reported to affect growth of peppers (*Capsicum annuum* L.) (Frankenberger and Arshad, 1991), cotton (*Gossypium hirsutum* L.) (Arshad et al., 1995), potato (*Solarium chacoense* x *Solatium acaule)* (Ahmad et al., 1999), corn (Ahmad et al., 2007), and snap beans (*Phaseolus vulgaris* L.) (El-Awadi et al., 2011). Furthermore, Dawood and Sadak (2007) reported that spraying plants with TRP increased plant growth and yield of the canola (*Brassica napus* L.) variety ‘Pactol’. Other researchers found that TRP applications improved growth parameters such as leaf number and shoot length, yield and fruit quality of orange ‘Obsek’ trees (*Citrus sinensis* L.) (Ahmed et al., 2012).

Sarwar and Frankenberger (1994) reported that TRP applied at the appropriate concentrations can have positive effects on certain corn growth parameters such as plant height, shoot weight and root growth comparable to application of pure auxins like tryptophol and indole-3-acetic acid. Frankenberger et al. (1990) also reported a significantly positive effect of soil applied TRP on radish (*Raphanus sativus*) growth and productivity.

Nitrogen is an essential element for animal and plants. In the latter, it plays extremely important roles as part of a number of organic molecules including chlorophyll, amino acids, proteins and enzymes. Even though the atmosphere contains about 78% N and cultivated soils contain between 1,350 and 6,700 kg N ha^{-1}, N is often regarded in agricultural production as the most limiting element (Näsholm et al., 2009; Robertson and Vitousek, 2009; Luce et al., 2011;

Sawyer, 2012; Xu et al., 2012). While in natural ecosystems free amino acids, peptides- and protein-bound amino acids make up the largest fraction of soil organic N, in agricultural systems NO_3^- and NH_4^+ are commonly found in higher concentrations than free amino acids due to addition of synthetic fertilizers and other agricultural management practices that influence soil N transformations (Olk, 2007; Jämtgård et al., 2010; Vinall et al., 2012).

Inorganic N forms are the preferable forms for uptake of N for plants; thus, mineralization of organic N is considered as a crucial step to achieve optimal plant N nutrition (Lipson and Näsholm, 2001; Fageria and Baligar, 2005; Havlin et al., 2005; Mikkelsen and Hartz, 2008; Farrel et al., 2013; Li et al., 2013). Nonetheless, direct absorption of soil amino acids by plants is a topic that has gained attention and is still a subject of debate (Näsholm, et al., 2000; Owen and Jones, 2001). Because modern high-yielding cropping systems rely heavily on N fertilizer inputs, their use has greatly increased during the last four decades. Along with the continued increase of synthetic N fertilizers, environmental concerns like air and ground water pollution have arisen as well.

The unquestionable benefits of N fertilizer utilization has made possible much of the large increase in the supply of food to the world's growing population. On the other hand, the negative implications of low N recovery by crop plants have led to environmental degradation related to serious water and air contamination issues (Tilman 1998; Tilman et al., 2001; Robertson and Vitousek, 2009; Sutton et al., 2011). Nitrogen losses are related to microbial transformations of N in soils. Leaching and denitrification are the main processes that lead to N losses in agricultural systems (Subbarao et al., 2012).

Soil application of amino acid biosynthesis byproducts from industrial processes may improve plant productivity by altering soil processes and plant physiological attributes when used as N sources or soil amendments in agricultural systems. The organic N in these amino acids byproducts may influence plant N metabolism and improve N use efficiency in crop plants. In a review article by Halpern et al. (2015), amino acids are classified as biostimulants, which are defined as substances capable of modifying physiological processes in plants.

Furthermore, the N in biosynthesis byproducts may be less vulnerable to loss than N in inorganic fertilizers, and that may decrease N exports to surface and groundwater bodies. The amino acids TRP and LYS are hydrophobic and polar positive charged, respectively. Both characteristics of these amino acids should make their N less susceptible to losses by leaching.

Amino acids may represent an alternative source of N for crop plants. Moreover, many studies and reviews have shown that numerous grass and tree species growing under managed or natural ecosystems can take up amino acids directly from soils (Schobert et al., 1988; Chapin, et al., 1993; Näsholm, et al., 2000; Lipson and Näsholm, 2001; Warren and Adams, 2007). Despite the considerable amount of evidence that plants absorb organic N from simple molecules such as amino acids or oligomers including di-, tri- and tetra- peptides (Schobert, 1988; Chapin et al., 1993; Näsholm, et al., 2000; Paungfoo-Lonhienne, 2012; Vinall et al., 2012; Farrel et al., 2013), the relevance of these organic molecules for N nutrition of plants remains unclear. Experiments using ^{15}N, confirmed that free amino acids are a readily available form of N for higher plants (Öhlund and Näsholm, 2004; Warren, 2006).

Although the majority of studies investigating amino acids as N sources have been carried out in natural ecosystems, amino acid uptake evidence in some crop plants has been demonstrated. Vinall et al. (2012) reported that sugar cane (*Saccharum officinarum* L.) has the ability to take up N from amino acids and that N supplied from amino acids produced similar biomass to N supplied by inorganic sources in axenic culture and pot studies. Similarly, in a growth chamber study, corn seedlings grown with high amino acid concentrations became better competitors for amino acids than the soil microbial community (Jones et al., 2005). Näsholm et al. (2000) reported that four commonly used pasture grasses in European grasslands were able to absorb organic N from the simple amino acid glycine under field conditions.

Concentration of amino acids in the soil solution was found to play a major role in plant amino acid uptake. But it remains unclear what are the optimum amino acid concentration levels in soils required for plant uptake. In this sense there is a wide range of amino acid concentrations that have been proposed as optimal and they vary significantly between and within plant species (Jämtgård, 2010). In a recent study, Hill et al. (2011) reported that wheat (*Triticum aestivum* L.) was able to take up and metabolize amino acids at rates comparable to those when inorganic N sources were utilized. Their findings suggest it is necessary to reconsider the current assumptions about plant available N forms. Another desirable characteristic of using amino acid biosynthesis byproducts for agricultural purposes is that their chemical composition includes other important nutrients in addition to N, including P, K, S and Fe (Martinez and Tabatabai, 1997).

Diversity in amino acid biosynthesis byproducts chemical composition depends upon the raw materials and the fermentation conditions used to produce the pure feed amino acids. For example Henning (2007) reported that biosynthesis byproducts are N-rich, but they also contain other nutrients at similar or higher levels than N as in the case of Cl and S for two LYS byproducts. High S and P concentration in biosynthesis byproducts was reported by Zhu et al. (1995), making them a potentially good soil amendment for agricultural soils. Others beneficial aspects of soil applied amino acids encompass improvement of soils chemical and physical characteristics, root growth and increase activity of NO_3^- assimilation enzymes (Garcia-Martinez et al., 2010; Walch-Liu et al., 2006; Halpern et al., 2015).

As the production of feed grade amino acids is steadily increasing, the production of their N rich biosynthesis byproducts is raising as well. This situation presents an opportunity to utilize these byproducts as a potential suitable N source for corn production. In this thesis (Chapter 2), we investigated the suitability of amino acid biosynthesis byproducts as a source of N for corn production. For this a field study was conducted during two continuous years in central Iowa, USA to evaluate the impacts of soil applied amino acid byproducts on corn productivity. Additionally, other parameters including corn growth and development, plant physiology and grain quality indicators were evaluated.

References

Ahmad, M., Pervez, A. M., Tahir, F. M., Anwar, U. H., 1999. Effects of L-tryptophan on the growth and yield of Potato cv. Pars-70. Int. J. Agr. Biol. 1, 30-32.

Ahmad, R., Khalid, A. H., Arshad, M., Zahir, A., Mahmood, T., 2007. Effect of compost enriched with N and L-tryptophan on soil and maize. Agron. Sustain. Dev. 28, 1-7.

Ahmed, H., Khalil, A. H., El-Rahman, M. K., Nadia, A. M., Hamed, A. M., 2012. Effect of zinc, tryptophan and indole acetic acid on growth, yield and chemical composition of Valencia orange trees. J. Appl. Sci. Res. 8, 901-914.

Ajinomoto Inc. 2004. FY 2003 market and other information. Retrieved from: https://www.ajinomoto.com/en/ir/pdf/q1_fy2004_data.pdf

Ajinomoto Inc. 2015. FY 2014 market and other information. Retrieved from: http://www.ajinomoto.com/en/ir/pdf/FY14_Data_E.pdf

Arshad, M., Hussain, A., Shakoor, A., 1995. Effect of soil applied L-tryptophan on growth and chemical composition of cotton. J. of Plant Ntr. 18, 317-329.

Arshad, M., Frankenberger, W. T. Jr., 1998. Plant growth-regulating substances in the rhizosphere: Microbial production and functions. Adv. Agron. 62, 45-151.

Bidwell, R. G., 1979. Plant Physiology, second ed. MacMillan, New York.

Chapin, E S., Moilanen, L., Kielland, K., 1993. Preferential usage of organic nitrogen for growth by a non-mycorrhizal sedge. Nature 361, 150-152.

Dawood, M., Mervat, G., Sadak, S., 2007. Physiological response of canola plants (*Brassica napus* L.) to tryptophan or benzyladenine. Lucrări Ştiinţifice 50, 198-207.

Deloitte, 2014. Opportunities for the fermentation-based chemical industry: An analysis of the market potential and competitiveness of North-West Europe. Deloitte, The Netherlands.

DeSutter, T. M., Cihacek, L. J., 2009. Potential agricultural uses of flue gas desulfurization gypsum in the northern Great Plains. Agon. J. 101, 817-825.

Economic Research Service, 2011. Fertilizer use and price. Retrieved from: Economic Research Service:

http://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx

El-Awadi, M. E., El-Bassiony, A. M., Fawzy, Z. F., El-Nemr, M. A., 2011. Response of snap bean (*Phaseolus vulgaris* L) plants to nitrogen fertilizer and foliar application with methionine and tryptophan. Nat. Sci. 9, 87-94.

Fageria, N. K., Baligar, V. C., 2005. Enhancing nitrogen use efficiency in crop plants. Adv. Agron. 88, 98-185.

FAO, 2004. Protein Sources for the animal feed industry. Proceedings of an expert consultation and workshop held at Bangkok. FAO, Rome.

Farrell, M., Hill, P. W., Farrar, J., Deluca, T. H., Roberts, P., Kielland, K., Dahlgren, R., Murphy, D., Hobbs, P.J., Bardgett, R. D., Jones, D. L., 2013. Oligopeptides represent a preferred source of organic N uptake: a global phenomenon. Ecosystems 16, 133-145.

Frankenberger, W. T. Jr., Chang A. C., Arshad, M., 1990. Response of *Raphanus sativus* to the auxin precursor L-tryptophan applied to soil. Plant Soil 129, 235-241

Frankenberger, W. T. Jr., Arshad, M., 1991. Yield response of *Capsicum Annuum* to the auxin precursor, L-tryptophan applied to soil. PGRSA Quart. 19, 231-240.

Frankenberger, W. T. Jr., Arshad M., 1995. Phytohormones in soil: microbial production and function, first ed. Marcel Dekker, New York.

García-Martínez, A. M., Díaz, A., Tejada, M., Bautista, J., Rodríguez, J., Santa María, C., Revilla, E., Parradoa, J., 2010. Enzymatic production of an organic soil biostimulant from wheat-condensed distiller solubles: Effects on soil biochemistry and biodiversity. Proc. Bioch. 45, 1127-1133.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., Yermiyahu, U., 2015. The use of biostimulants for enhancing nutrient uptake. Adv. Agron. 130, 141-174.

Havlin, J. L., Tisdale, S. L., Beaton, J. D., Nelson, W. L., 2005. Soil fertility and fertilizers. Pearson Education, Inc., Upper Saddle River, NJ.

Henning, S., 2007. Corn and soil test response to by-Product nitrogen sources. Iowa State Research Farm Progress Reports. Paper 733.

Hill, P. W., Quilliam, R. S., DeLuca, T. H., Farrar, J., Farrell, M., Roberts, P., Newsham, K. K., Hopkins, D. W., Bardgett, R. D., Jones, D. L., 2011. Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. PLoS One 6, 1-4.

Huang, S., Adams, W. R., Zhou, Q., Malloy, K. P., Voyles, D. A., Anthony, J., Kriz, A. L., Luethy, M. H., 2004. Improving nutritional quality of maize proteins by expressing sense and antisense zein genes. J. Agric. Food Chem. *52*, 1958–1964.

Huang, S., Frizzi, A., Florida, C. A., Kruger, D. E., Luethy, M. H., 2006. High lysine and high tryptophan transgenic maize resulting from the reduction of both 19- and 22-kD α-zeins. Plant Mol. Biol. 61, 525-535.

Ikeda, M., 2003. Amino acid production processes, in: Scheper, T., Daurie, R., Thommel, J. (Eds), Microbial production of L-amino Acids. Springer, New York, 79, pp 1-34.

Jämtgård, S. Näsholm, T.,Huss-Danell, K., 2010. Nitrogen compounds in soil solutions of agricultural land. Soil Biol. Chem. 42, 2325-2330.

Jones, D. L., Shannon, D., Junvee-Fortune, T., Farrar, J. F., 2005. Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biol. Biochem. 37, 179-181.

Khalid, A., Arshad, M., Zahir, Z. A., Khalid, M., 2001. Relative efficiency of rhizobacteria for auxin biosynthesis. J. Biol. Sci. 1, 750-754.

Khalid, A., Arshad M., Zahir A., 2006. Phytohormones: microbial production and applications, in Uphoff, N., Ball, A. S., Fernandes, E., Herren, H., Husson, O., Laing, M., Palm, C., Pretty, J., Sanchez, P., Sanginga, N., Thies, J. (Eds), Biological approaches to sustainable soil systems. Taylor & Francis, Florida, pp 207-22.

Leuchtenberger, W., Huthmacher, K., Drauz, K., 2005. Biotechnological production of amino acids and derivatives: current status and prospects. Appl. Microbiol. Biot. 69, 1–8.

Li, S., Wang, Z., Stewart, B. A., 2013. Responses of crop plants to ammonium and nitrate N. Adv. Agron. 118, 205-397.

Lipson, D., Näsholm, T., 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128, 305-316.

Luce, M., Whalen, J. K., Ziadi, N., Zebarth, B., 2011. Nitrogen dynamics and indices to predict soil nitrogen supply in humid temperate soils. Adv. Agron. 112, 56-102.

Martens, D. A., Frankenberger, W. T. Jr., 1993. Metabolism of tryptophan in soil. Soil Biol. Chem. 25, 1679-1687.

Martinez, C. E., Tabatabai, M. A., 1997. Decomposition of biotechnology by-products in soils. J. Environ. Qual. 26, 625-632.

Mikkelsen, R., Hartz, T. K., 2008. Nitrogen sources for organic production. Better crops 92, 16-19.

National Agricultural Statistics Service, 2011. Acreage. Retrieved from: USDA: National Agricultural Statistics Service: http://www.nass.usda.gov/Newsroom/2013/06_28_2013.asp

Näsholm, T., Huss-Danell, K., Högberg, P., 2000. Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81, 1155-1161.

Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. New Phytol. 182, 31-48.

Olk, D. C., 2007. Improved analytical techniques for carbohydrates, amino compounds, and phenols: tools for understanding soil processes. Soil Sci. Soc. Am. J. 72, 1672-1682.

Owen, A. G., Jones, D. L., 2001. Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biol. Biochem. 33, 651-657.

Öhlund, J., Näsholm, J., 2004. Regulation of organic and inorganic nitrogen uptake in scots pine (*Pinus sylvestris*) seedlings. Tree Physiol. 24, 1397-1402.

Paungfoo-Lonhienne, C., Visser, J., Thierry, G. A., Schmidt, S., 2012. Past, present and future of organic nutrients. Plant Soil 359, 1-18.

Robertson, P. G., Vitousek, P. M., 2009. Nitrogen in Agriculture: balancing the cost of an essential resource. Ann. Rev. Environ. Resour. 34, 92-125.

Sarwar, M., Frankenberger, W. T. Jr., 1994. Influence of L-tryptophan and auxins applied to the rhizosphere on the vegetative growth of *Zea mays* L. Plant Soil 160, 97-104.

Sawyer, J. E., 2012. Soil Nitrogen Cycle. Iowa State University. Presentation at the Climate and corn-based cropping systems coordinated agricultural project (CSCAP) 2012 Annual Meeting.

Schobert, C., Köckenberger, W., Komor, E., 1988. Uptake of amino acids by plants from the soil: A comparative study with castor bean seedlings grown under natural and axenic soil conditions. Plant Soil 109, 181-188.

Scott, M. P., Bhatnagar, S., Betran, S., 2004. Tryptophan and methionine levels in quality protein maize breeding germplasm. Maydica 49, 303-311.

Sims, J.T., Stehouwer, R. C., 2002. Recycling of nitrogen through land application of agricultural, municipal, and industrial by-products, *in:* J. Schepers (ed.) New edition *of Nitrogen in Agricultural Soils,* Agronomy Series 22.

Subbarao, G. V., Sahrawat, K. L., Nakahara, K., Ishikawa, T., Kishii, M., Rao, I. M., Hash, C. T., George, T. S., Rao, S., Nardi, P., Bonnett, D., Berry, W., Suenaga, K., Lata, C., 2012. Biological nitrification inhibition - A novel strategy to regulate nitrification in agricultural systems. Adv. Agron. 114, 249-302.

Sutton, M., Oenema, O., Erisman, J. W., Leip, A., Grinsven, H., Winiwarter, W., 2011. Too much of a good thing. Nature 472, 159-161.

Tilman, D., 1998. The greening of the green revolution. Nature 396, 211-212.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D., Swackhame, D., 2001. Forecasting agriculturally driven global environmental change. Nature 5515, 281-284.

Vinall, K., Schmidt, S., Brackin, R., Lakshmanan, P., Robinson, N., 2012. Amino acids are a nitrogen source for sugarcane. Funct. Plant Biol. 39, 503-511.

Walch-Liu, P., Liu, L. H., Remans, T., Tester, M., Forde, B.G., 2006. Evidence that L-glutamate can act as an exogenous signal to modulate root growth and branching in Arabidopsis thaliana. Plant Cell. Physiol. 47, 1045–1057.

Warren, C. R., 2006. Potential organic and inorganic N uptake by six Eucalyptus species. Funct. Plant Biol. 33, 653-660.

Xu, G., Fan, X., Miller, A., 2012. Plant nitrogen assimilation and use efficiency. Ann. Rev. Plant Biol. 63, 153-182.

Zhu, B., Tabatabai, M. A., Henning, S. J., 1995. Biotechnology by-products as sources of nitrogen for plants. J. Environ. Qual. 24, 986-992.

CHAPTER 2. AMINO ACID BIOSYNTHESIS BYPRODUCTS ARE A SUITABLE SOURCE OF NITROGEN FOR CORN PRODUCTION

A paper submitted to Fields Crop Research

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Abstract

Commercial biosynthesis of the amino acids tryptophan (TRP) and lysine (LYS) results in byproducts that are rich in both organic and inorganic compounds. Their nitrogen (N) content may have value as fertilizer N replacement in corn (*Zea mays* L.) production. We conducted a two-year field study where soil applied pure TRP, its biosynthesis byproduct and LYS byproduct were evaluated as replacements for synthetic N fertilizers in corn production. The experimental treatments were eight isonitrogenous N formulations applied at 196 kg N ha^{-1} in a randomized complete block with four replicates. The N treatments were ammonium nitrate (AN) and ammonium sulfate (AS) as controls; different levels of pure TRP replacing NH_4-N from AN, and combinations of both TRP and LYS byproduct at different levels. In-season plant measurements leaf area index (LAI), normalized difference vegetation index (NDVI), grain quality (grain protein and oil concentration) and total N accumulation were not affected by N treatments. Additionally, TRP and LYS biosynthesis byproducts did not influence corn grain yield, though some of the byproducts treatments had significantly greater harvest index (HI) relative to control treatments. Our results also highlight the enhancement in grain N partitioning when biosynthesis byproducts are used relative to controls treatments, suggesting that N from amino acids may be influencing the physiology of N partitioning. Biosynthesis byproducts from TRP and LYS production can replace AN and AS fertilizers on an equivalent N basis to produce high corn grain yields.

Keywords: Corn; Tryptophan; Biosynthesis byproducts; Lysine; Nitrogen fertilizers; Corn production, LAI; NDVI; SPAD; Amino acids.

1. Introduction

Nitrogen (N) from inorganic fertilizers has been recognized as a key factor for continued high yields and yield improvements (Peoples et al., 1995; Cassman et al., 1998; Tilman et al., 2002; Duvick, 2005). Modern grain production systems rely heavily on external inputs, with N from inorganic fertilizers one of the major drivers for the high productivity of these systems (Tilman, 1998; Grassini et al., 2015) and fertilizer N is the most widely applied nutrient in agricultural production systems in the US. In 2010, about 60% of the total fertilizers applied to the US farmland was N, with application of about 13 x 10^6 t of synthetic N fertilizer, with corn receiving 46% of the total N used in 2011 (ERS, 2011). Iowa typically has the largest amount of land planted to corn and the greatest average yield. In 2013, the Iowa corn crop represented roughly 14% of the total land planted for corn production in the US (NASS, 2013).

The majority of nitrogenous fertilizers are applied either in NH_4^+ (e.g., anhydrous ammonia, ammonium nitrate, ammonium sulfate) or urea forms worldwide (IFA, 2015). Organic N is the most abundant form of N in agricultural soils, but few crop plants are able to utilize organic N directly. Instead, NH_4^+ and NO_3^- are the major N sources for crop production systems (Havlin et al., 2005; Mikkelsen and Hartz, 2008). Although these two forms of inorganic N represent the sources of N taken up by crop plants, their presence in agricultural soils is below that required for intensive grain crop production (Cassman et al., 2002; Sawyer, 2012).

Amino acids may represent an alternative source of N in agricultural systems. However, until recently it was debated whether soil applied amino acids are taken up as NO_3^- or NH_4^+ or in organic forms as simple amino acids by plants (Näsholm et al., 2000; Owen and Jones, 2001). Currently, there is an increasing awareness that amino acids can be taken up by crop plants (Schobert et al., 1988; Chapin et al., 1993; Warren and Adams, 2006). Moreover, experiments using ^{15}N, confirmed that amino acids are a readily available form of N for higher plants (Öhlund and Näsholm, 2004; Warren, 2006). However, there is a paucity of information available regarding the effectiveness of soil applied amino acids under field conditions as a source of N on crop productivity, growth and development.

Currently amino acids are used as additives in feed to enhance the limited quality of the corn-based-diets for monogastric diets. The amino acids TRP and LYS are produced by bacterial fermentation. Feed amino acid production worldwide has increased steadily during the last decades, as TRP, LYS and threonine the key products of the fermentation industry. Biosynthesis production of TRP increased from around 1 thousand in 2003 to 14 thousand metric tons in 2013, while LYS production climbed from 700 thousand to 2100 thousand metric tons during the same time period (Ajinomoto Inc., 2004 and 2015). As in the majority of industrial processes, byproducts are produced from the amino acid biosynthesis process. Currently, these byproducts are underutilized. However, the utilization of these biosynthesis byproducts may provide many benefits when used on agricultural land. Residual biosynthesis products contain amino acids and other forms of N like $NH4^{+}$-N and could potentially replace at least some N fertilizer (Henning, unpublished, 2007; Singer et al., 2007). Some amino acids as TRP are precursors for auxin biosynthesis in plants and soils, which may result in positives corn physiological responses. This could be another important benefit of amino acid byproduct utilization on cropland. In addition, amino acids products and byproducts contain other plant nutrients (K, Ca, S, Fe) that may be plant available and benefit corn growth and productivity.

The use of amino acids has been addressed by several studies focused on their use as plant hormone precursors for both soil microorganisms and plants. The effectiveness of soil- and foliar- applied TRP on growth and yield has been demonstrated with several crops, including watermelon (*Citrullus Lanatus L*.), pepper (*Capsicum annuum* L.), cotton (*Gossypium hirsutum* L.), radish (*Raphanus sativus* L.) and snap beans (*Phaseolus vulgaris* L.) (Frankenberger et al., 1990; Frankenberger and Arshad, 1991; Frankenberger and Arshad, 1991b; Arshad et al., 1995; El-Awadi et al., 2011). In a glasshouse experiment, Sarwar and Frankenberger (1994) compared the effect of applied TRP with that of known auxins (indole-3-acetamide, tryptophol and indole-3-acetic acid) on corn growth and development parameters and found that TRP had a comparable or better effect on corn growth than the tested auxins. The majority of these studies were conducted under controlled conditions (e.g., glasshouses and greenhouses trials), and very few experimental field trials have been conducted that demonstrate positive effects of amino acids applications on grain crop growth, development or yield.

In a series of publications, a LYS byproduct and other byproducts generated from the biotechnology industry were evaluated to determine their potential as either nutrient sources for corn or as soil amendments. In a greenhouse study Zhu et al. (1995), demonstrated that various biotechnology byproducts are good potential sources of N for corn production in Iowa, with LYS and glutamate byproducts having the greatest potential. Subsequently a laboratory study determined that different biotechnology byproducts including LYS are useful as soil amendments due to their high content of soil enzymes and organic C (Martinez and Tabatabai, 1997). In addition Henning (unpublished, 2007) in a one year field study reported that two LYS biosynthesis byproducts could be used as N sources since corn grain yields were not negatively affected. However, the use of these materials may acidify soils.

Iowa has a large amino acid production industry that produces substantial amounts of biosynthesis byproducts that contain both N and residual amino acids. We conducted a field study to determine whether TRP or LYS biosynthesis byproducts could replace fertilizer N for corn production.

2. Materials and methods

2.1. Field sites and experimental design

Field experiments were conducted to evaluate corn growth and yield responses to application of N sources including two commercial fertilizer, an amino acid product and two amino acid byproducts biosynthetically produced. Trials were conducted during two consecutive growing seasons (2013 and 2014) at three sites in central Iowa near Ames. The study was conducted on adjacent fields at the Iowa State University Sorenson Research Farm (42°01' N; 93° 46' W) in 2013 (hereafter referred to as SF I) and 2014 (hereafter referred to as SF II), respectively. In 2014, an additional site was included at the Agricultural Engineering and Agronomy Research Farm (42°00' N; 93°44' W) (hereafter referred to as AF). At SF I, the predominant soil was a Clarion loam (fine-loamy, mixed, superactive, mesic Typic Hapludolls) (NRCS, 2015), while at SF II soil was a Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) (NRCS, 2011). In 2014 at AF, the predominant soil type was Nicollet loam. The three locations were cropped with soybean [*Glycine max* (L.) Merr.] the prior year. Annual and historic data on precipitation and temperature were collected and downloaded from an automated weather station (NWS COOP 8WSW) located within 3 km of the study area (Iowa Environmental Mesonet Network, 2014). Growing degree days (GDD, 10°C base temperature and 30°C as T_{max}) were calculated right after corn planting as:

$$GDD = \sum [(\text{daily maximum temp.} + \text{daily minimum temp.})/2] - 10. \quad [1]$$

The experimental design was a randomized complete block (RCBD) with eight isonitrogenous N sources (T1 – T8) and four replications. The alternative N sources included TRP (Feed grade; 98% pure L-TRP), TRP byproduct and LYS byproduct, obtained from a bio-fermentation plant (Ajinomoto North America Inc., Eddyville, Iowa) and two commercial N fertilizers that served as control treatments. The TRP was a very fine white crystalline powder, whereas both byproducts were suspensions. The control treatments consisted of ammonium nitrate (AN) (hereafter referred to as "T1"), and ammonium sulfate (AS) (hereafter referred to as “T8”) (Table 1). Another treatment with a single N source was TRP byproduct referred as “T5” hereafter. The remaining five treatments were combinations of two N sources as follows: (i) T1 + TRP (7% of N supplied by TRP) referred as “T2” hereafter, (ii) T1 + TRP (14% of N supplied by TRP) referred as “T3” hereafter, (iii) T1 + TRP (30% of N supplied by TRP) referred as “T4” hereafter, (iv) T5 + LYS byproduct (50 and 50% contributed toward the standard targeted N rate, respectively) referred as “T6” hereafter and (v) T5 + LYS byproduct (24 and 76% contributed toward the standard targeted N rate, respectively) referred as “T7”.

The amount of TRP and amino acid byproducts varied depending on N treatment. For T2, T3 and T4 the TRP was applied at 100, 200 and 410 kg ha^{-1}, respectively. In T6, TRP and LYS byproducts were applied at 14.2 and 1.4 t ha^{-1}, respectively; while in T7, TRP and LYS byproducts were applied at 8.0 t ha^{-1} and 2.1 t ha^{-1}, respectively to reach the targeted N application rate of 196 kg ha^{-1}.

Before planting, soil samples were taken from the 0- to 15-cm depth with a 2.2-cm diameter hand probe. Soil samples were composites of twelve soil cores collected randomly from within each study site. Samples were air dried and analyzed for Mehlich-3 P and K, pH, organic matter and nitrate-N at the Iowa State University Soil and Plant Analysis Laboratory. The pH was measured on a 1:1 ratio of soil and water using a pH meter (Warncke and Brown, 1998). Total organic carbon (TOC) was determined using dry combustion (Matejovic, 1997) with a LECO CHN-2000 (LECO Corporation, St. Joseph, MI) and converted to organic matter (OM) (Table 2).

According to the Iowa State University soil-test interpretations (Mallarino et al., 2013) available P levels were low at both SF I and SF II, whereas they were very high at AF. The available K soil levels were between optimum and very high across locations and years (Table 2). Fertilizers were broadcast uniformly with a Gandy Model 62 Series air-delivery fertilizer system (Gandy Company, Owatonna, MN) across the entire study before corn planting at 67 kg P ha^{-1} and 90 kg K ha^{-1}. A few days before application, amino acid byproducts chemical characteristics were determined from a 2-L composite sample. The TRP sample for chemical analyses was obtained from a commercial bag of TRP.

Individual plot size was 6.0 m long x 3.1 m wide with four corn rows per plot on 0.76-m row spacing. Treatments were designed to supply N either from sole or combined N sources as described previously. All treatments were adjusted to a single N rate of 196 kg N ha^{-1}. Seedbed preparation was done using a rotary tiller at 15- to 20-cm depth just before fertilizer treatment applications. The alternative N sources were broadcast applied by hand and both dry and liquid treatments were applied pre-planting and immediately after planting, respectively. The AN and AS were weighed and applied with a 1.5 m-wide Gandy spreader (Model 6500, Gandy Co., Owatonna, MN) pre-plant and incorporated immediately following application with a field cultivator. Corn was planted at 79,000 seeds ha^{-1} the third week of May in 2013 and 2014 with a full-season hybrid adapted to central Iowa (DuPont Pioneer '33W84', 111-day relative maturity) with a four row Kinze 3000 pull type planter (Kinze, Williamsburg, IA). Pre-emergence application of acetochlor [(2-chloro-2-methyl-6-ethyl-N-phenyl)-N-ethoxymethylacetanilide] at 2 kg a.i. ha^{-1} was used both years for weed control.

2.2. In-season-plant measurements

Morphological and physiological measurements were taken on a weekly basis over seven consecutive weeks, as weather permitted, at each research site. Measurements were taken at 853, 1031, 1200, 1398, 1473, 1635 and 1743 GDD after planting at SF I (V8, V11, V13, VT, R1, R2 and R3, respectively); at 672, 859, 974, 7097, 1261, 1367 and 1489 GDD at both research sites in 2014 (V8, V10, V12, V15, VT, R1 and R2, respectively), resulting in seven measurements dates for each study environment. The four plant parameters were acquired on the same day of each measurement date. All measurements were collected from the center two rows of each four-row plot to minimize border effects.

Corn phenology was determined in each plot from emergence to maturity using the Leaf Collar Method (Abendroth et al., 2011). Mean plot growth stage was based on twelve plants per plot, six in row two and six in row three, that were tagged and staged weekly. Phenology during reproductive growth stages was assessed by peeling back husks to view kernel development.

Corn leaf greenness was estimated with a SPAD meter (Minolta SPAD-502, Konica Minolta, Osaka, Japan) (Schepers et al., 1992; Markwell et al., 1995). The SPAD meter produces an output in SPAD units that is correlated with leaf chlorophyll and N concentration (Schepers et al., 1992; Markwell et al., 1995).Readings were taken on the same 12 plants in each plot utilized for corn phenology and averaged. During vegetative growth readings were taken from the uppermost fully expanded leaf with a visible collar. From tasseling onward, readings were taken on the ear leaf. Measurements were taken midway between the stalk and the tip, and midway between the midrib and leaf margin (Peterson et al., 1993).

Leaf area index (LAI) was estimated with a Decagon AccuPAR (Decagon Devices Inc., Pullman, WA) in each plot. Four measurements were taken in each plot diagonally across the two central rows below the canopy between 0900 and 1300 h. The mean of these four readings was used as the plot LAI.

Corn canopy biomass and growth was measured with a Crop Circle ACS-210 optical, active canopy sensor (Holland Scientific, Lincoln, NE). Sensing was conducted between 0900 and 1300 h on cloudless days. The sensor was mounted on a hand-held mast, positioned mid inter-row, and carried through the middle of each plot at 0.6-0.9 m above the corn canopy. Reflectance data were collected at a sample output rate of 6 Hz (Holland Scientific, 2004), giving 50 to 70 readings per plot. Plot mean normal difference vegetative index (NDVI) was automatically calculated from reflectance data produced with the internal software in the Holland Scientific GeoScout GLS-420 datalogger (Holland Scientific, 2006) as:

$$NDVI = (NIR – VIS) / (NIR + VIS) \quad [2]$$

where NIR is the fraction of emitted infrared light (880-nm) returned from the corn canopy and VIS is the fraction of emitted visible light (590-nm) returned from the corn canopy (Gitelson et al., 1996).

2.3. N concentrations, uptake and harvest index and corn production and grain quality

At corn physiological maturity (R6) a six-plant sample was collected to determine N concentrations in stover, cobs and grain, and to calculate harvest index (HI) and N uptake following the procedures of Dobermann (2005). Six randomly selected plants from the two middle rows were cut at the soil surface and separated into two components: stover (leaves, husks and stalks) and ears. The stover fresh fraction was weighed and a sub-sample of three plants was chopped into small pieces with a machete for further processing. The obtained six-plant sample fresh weight served for later final total stover yield estimation. The chopped stover subsamples were weighed and placed in a forced-air oven at 70°C until constant weight was reached to determine moisture content, stover dry weight and obtain a sample for chemical analysis. These representative corn stover sub-samples along with the cobs samples were further ground to pass a 2-mm sieve using a Wiley mill (Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ). Subsamples were sent to the Iowa State University Soil and Plant Analysis Laboratory in Ames, IA for total C and N analysis by dry combustion (Model CHN-2000 C-N analyzer, LECO Corp., St. Joseph, MI). Corn stover, grain and cob N uptake were calculated as products of N concentration in each plant fraction and their respective dry matter accumulation values. Total corn aboveground N uptake was the sum of stover, grain and cob N uptake. Nitrogen harvest index (NHI) represents the proportion of grain N in total aboveground biomass N and was calculated as:

NHI = UG (100% dry matter)/UT [3]

where UG is grain N uptake (kg ha^{-1}) and UT is total aboveground N (kg ha^{-1}) (Sindelar, 2015). The six-ear fraction collected at R6 were weighed and then shelled. The weights of cobs and grain were determined separately. A grain subsample was taken from the six-ear samples, dried and reweighed as previously described for stover samples. The six-plant cobs samples were processed as previously described for the grain subsamples. Dry weights were used to calculate stover yield and HI for each treatment. The HI was calculated as:

HI = Grain weight (100% dry matter) / (total aboveground biomass) [4]

Corn grain yield was determined from the two center rows two weeks after R6 sample plants were collected by hand harvesting the entire length of these rows. Corn harvest plant population was determined by counting all the plants in the two middle rows of each plot. Ears were hand-picked and then fresh ear weight determined from each plot. Eight cobs were randomly sampled and shelled; shelling percentage was obtained from the eight shelled cobs by dividing fresh grain weight by fresh cob (including grain) weight. Moisture was measured from the eight shelled ears with a grain analysis computer (Model GAC2000, Dickey John, Auburn, IL). Field weight, shelling percentage and HI were used to calculate final grain yield for each plot. Stover yield and aboveground dry matter measurements were calculated at 100% dry matter. Final grain yield was adjusted to 155 g kg^{-1} moisture content.

Air dried whole corn kernels collected at harvest were used for grain quality analysis for protein and oil concentration at the Iowa State Grain Quality Laboratory, Ames, IA. Analyses were performed using near-infrared spectroscopy (NIRS) with a Foss Infratec-1225 near-infrared analyzer (Foss North America, Eden Prairie, NN) using Iowa State University calibration CN201301 for moisture, oil and protein concentrations. Corn protein and oil concentration results were expressed on a dry matter basis. Grain protein concentration was converted to grain N concentration by dividing protein concentration by a factor of 6.25.

2.4. Data analysis

Data were analyzed by analysis of variance using PROC MIXED in SAS version 9.3 (SAS Institute, 2010). Before analysis all data were checked to verify assumptions for independence and normality. In-season plant measurements were analyzed as a split-plot in time design (measurement dates were treated as repeated measurements), while corn production and grain quality parameters were analyzed as a RCBD. Mean separations were performed using appropriate t-tests (SAS Institute, 2010) when N sources, measurement date or interactions were significant. Differences were declared significant at the $P \leq 0.05$ level. For environments in the split plot in time where the main effects of N treatments, measurement date or the interaction between N treatments and measurement dates were significant, the slice option of the LSMEANS statement (SAS Institute, 2010) was used to assess the response of N treatments within each measurement date. For pairwise comparisons among N treatments the PDIFF option of the LSMEANS statement was used for both the split plot in time and the RCBD design. For the first the pairwise comparisons between N treatments were made within each measurement date and for the latter design the pairwise comparisons were made between N treatments across study environments. Environment and N sources were considered fixed factors, whereas block was considered random. Due to unbalanced years at each location, years and location were combined and analyzed as three separate environments. The LAI, SPAD and NDVI data were initially evaluated as a combined analysis; however, when the interaction of environment × N source was significant, environments were presented individually.

3. Results and discussion

3.1. Temperature and precipitation

Rainfall was excessive early in the growing seasons of 2013 and 2014 and delayed planting (Fig. 1). One replicate was lost at the SF I site following severe rainfall that washed out planted corn seed. Accumulated precipitation (from May through October) was 866 mm in 2014, 220 mm greater than the 20-yr historic mean of 646 mm, whereas in 2013 accumulated precipitation (431 mm) was lower than normal. In 2013 the period from July to September received substantially less than normal precipitation. Conversely, the period from June through

October (except July) in 2014 was unusually wet. Average air temperature in 2013 and 2014 did not vary greatly from the historic 20-yr average during this study, except for a slightly cooler than normal July in 2014 (Fig. 1).

3.2. Amino acid products and byproducts characteristics

Nutrient composition and concentration of the alternative N sources utilized were consistent across years; therefore chemical characteristics were pooled as presented in Table 3. On average the byproducts contained large amounts of N, P, K and S, while the TRP product had a significant amount of N. The C:N ratios of the three alternative N sources were below 5:1. Previous studies have indicated that materials with C:N ratios of less than 20:1 do not immobilize N (Mathur et al., 1993). Therefore, N immobilization in amino acids and their byproducts in this study would not be expected.

All alternative N sources in this study were acidic (pH <5) (Table 3). The N concentration differed considerably among alternative N sources. As expected, TRP had the highest total N concentration in relation to both byproducts, but LYS byproduct composition had about 12-fold higher total N compared with TRP byproduct. In spite of the large difference in N concentration both byproducts were similar to N concentration values found in animal manures such as different poultry litters, swine manure and organic manures, but the N concentration in TRP was greater than those reported values for manure (Edwards and Daniel, 1992; Loecke et al., 2004; Ruiz Diaz and Sawyer, 2008; Diaz and Sawyer, 2012; Woli et al., 2012). The forms of N varied in the three alternatives N sources. Only in LYS byproduct was most of the N in the form of NH_4^+; in TRP and TRP byproduct organic N was the main fraction of the total N (> 80% of total N). The NO_3^- concentration for all alternative N sources was negligible (Table 3).

All the alternative N sources used were high in sulfur (S) concentration except TRP. The two amino acids byproducts differed in their S concentration. The S concentration of LYS byproduct was nearly 17-fold greater than that found in TRP byproduct, likely due to biosynthetic or purification process differences between production of these two amino acids. Interestingly, LYS byproduct contained slightly more S concentration than total N. For treatments where LYS byproduct was the N source along with TRP byproduct, T6 and T7, the levels of S fertilization were 188 kg S ha^{-1} and 207 kg S ha^{-1}, respectively. For T5 where TRP byproduct was the sole N source the S fertilization was 149 kg S ha^{-1} (Table 4). Corn producers in IA have reported S deficiencies in recent years. Producers may benefit from land application of amino acid byproducts. In a recent extension publication S fertilizer study was conducted over S deficiencies soils in northeast Iowa. In this study application of 45 kg S ha^{-1} consistently increased corn grain yields at an average rate of 2.4 t ha^{-1} in comparison to non S applied soils (Sawyer, 2009). Some crops like canola are particularly sensitive to low S soil availability (Franzen and Lukach, 2007). North Dakota is the leading producer of canola in the US with roughly 0.5 million hectares under canola production with steady increases in acreage projected (US Canola Association, 2015). Utilization of amino acids or their biosynthesis byproducts for canola production may enhance seed and oil production.

The two amino acids byproducts applied contained P and K, but concentrations were different in the two materials. LYS byproduct had greater P and K concentration relative to TRP byproduct. Where TRP byproduct was used as the sole N source in treatment T5, application rates were 33 kg P ha^{-1} and 62 kg K ha^{-1}. For treatments where N sources where combined (TRP byproduct +LYS byproduct) the P and K supplied was 21 kg P ha^{-1} and 41 kg K ha^{-1} in T6 and 15 kg P ha^{-1} and 30 kg K ha^{-1} in T7 (Table 4). In 2013, corn grain yield in Boone County, IA averaged 9.7 t ha^{-1} (NASS, 2014), which would have removed an estimated 31 kg P ha^{-1} yr^{-1}and 21 kg K ha^{-1} yr^{-1} (Mallarino et al., 2013). This indicates that annual applications of amino acids byproducts at rates sufficient to meet N corn requirements could also supply sufficient P and K for corn production. The rates of 67 kg P ha^{-1} and 90 kg K ha^{-1} applied to all plots in this study where intended to prevent any confounding effect on corn growth and yield due to the described P and K content of the used biosynthesis products. All the previously noted amino acids and byproducts characteristics have implications for corn performance as will be discussed further when corn growth and yield parameters are presented.

The industrial origin of the amino acids byproducts make them very consistent in terms of nutrient composition. This consistency in chemical composition and concentrations is a desired product characteristic when promoting the use of alternative nutrient sources. Consistent nutrient concentrations enables farmers to rely on nutrient management recommendations, making easier the practical use of new alternatives nutrient sources. Conversely, it is well documented that animal manures, which represent the most common alternatives nutrient sources, vary tremendously in their nutrient composition due to differences in animal feed, bedding, animal age and other factors (Chadwick et al., 2000).

Though we did not focus on soil effects due to biosynthetically produced amino acids land application, land applied amino acids and their byproducts could produce beneficial effects on soil properties due to their rich nutrient composition and organic C content. Martinez and Tabatabai, **(**1997**)** showed that various biosynthesis byproducts (including a LYS byproduct) are useful as soil amendments in incubated soil amendments. For this reason, further studies should evaluate the impact of using corn-derived amino acids on other physical and biological soil properties. Moreover, the high concentrations of P, K and S in the alternative N sources utilized in this study suggest a high likelihood of increasing productivity in agroecosystems characterized by low nutrient levels. Therefore, additional work is required to examine byproduct N from industrial amino acids production as a way to possibly improve corn productivity and fertility of nutrient deficient soils.

3.3. Grain yield

Grain yield was not influenced by N treatment, environment, or the N treatment × environment interaction (Table 5). Overall, N treatments produced similar corn grain yields, averaging 12.2 t ha^{-1} across study environments. Our results are in agreement with the findings of Henning (unpublished, 2007), who also reported no negative impacts on corn grain yield due to soil applied LYS. We believe that this lack of response may be associated with the persistent heavy rainfalls that occurred both years early in the corn season.

Prolonged saturation exacerbates nitrate losses and corn plants can become N deficient (Sawyer, 2014). Applications of N early in the season coupled with saturated soils reduce corn yield potential due to N loss by different mechanisms, including nitrate leaching and denitrification (Jones and Gunther, 2014; Nielsen, 2006; Kipling et. al., 2003; Randall et. al., 2003; Torbert et. al., 1993). Despite the excessive early season rainfall both years and unseasonably wetter than normal June in 2014, average corn grain yield both years was similar to the average grain yield reported for central Iowa in years where accumulated rainfall was near to long-term average precipitation. Average corn grain yields in Boone, IA, in 2007 and 2009 were 11.4 t ha^{-1} and 11.5 t ha^{-1}, respectively (NASS, 2014).

Effectiveness of soil applied TRP and LYS as a source of nutrients for corn grain production has been poorly documented. Ahmad et al. (2007), in a field study conducted in Pakistan where soil applied compost was enriched with TRP found that the enriched compost increased corn grain yield by around 21% in a sandy clay loam soil. Our results suggest that corn grain yields were as good as when AS and AN were used due to the readily available form of N in the utilized biosynthesis byproducts. Moreover, all treatments received the same N rate; strengthening our hypothesis about sources having similar N availability. This nutrient availability component is a key factor to consider when replacing traditional fertilizers with alternative nutrient sources. In the US cropland manure is one of the most common alternatives nutrients sources utilized (USDA, 2009). The amount of nutrients that becomes available for crop uptake after application of animal manures can be uncertain and research has shown diverse estimates of N availability. Some studies have shown that N readily available for plant uptake is about half of the total N applied the first year (Leikam et al., 2003; Motavalli et al., 1989). Other studies have demonstrated that more than 50 % of the total N applied is crop available, whereas others reported less than 50% is crop available within the first year after upon application to soil (e. g. Bitzer and Sims, 1988; Chadwick et al., 2000). This crop-availability limitation is often overcome by increasing the manure N application rate, which can exacerbate environmental concerns due to greater N losses and over application of P (Lithourgidis, 2007).

Land application of amino acid biosynthesis byproducts could be an important means of utilizing these materials, decreasing the cost for the industry associated with the management of these residues. Furthermore, the creation of new value-added products for the bio-fermentation sector may emerge if the biosynthesis byproducts utilized in this study are adopted by corn farmers for grain production.

3.4. Stover yield

In contrast to grain yields there was a significant main effect of N treatment on corn stover production when averaged across environments ($P < 0.05$). Also, corn stover yields varied significantly between study environments ($P < 0.01$); however, the N treatment response was consistent across environments as indicated by a nonsignificant N treatment × environment interaction ($P < 0.05$) (Table 5). For this reason, mean stover yields for the three environments are presented in table 5.

Stover yields were slightly lower than grain yields. Treatment T5 had the lowest yield (8.5 t ha^{-1}) and T1 the highest (9.7 t ha^{-1}). The T6 (9.0 t ha^{-1}) and T8 (9.3 t ha^{-1}) treatments produced stover yields comparable to T1. Stover production of these three N treatments was superior to the other five N treatments (Table 5). At SF I, stover yield was 27% and 37% lower than at SF II and AF, respectively (results not presented). Stover yields were similar to those reported in others corn-soybean production systems in central Iowa (Wilson et al., 2013; Karlen et al., 2011). If stover production is not a desired outcome then these byproducts have potential to decrease stover. However, decreased return of stover C to soil may result in long term decreases in soil organic matter (Johnson et al., 2006; Villamil et al., 2015)

3.5. Total biomass

Total dry matter (TDM) did not differ for N treatment and no N treatment × environment interaction occurred; however, the effect of environment on TDM was significant (Table 5). At SF I, TDM was 14% and 20% lower than SF II and AF, respectively (results not presented). Across N treatments and environments TDM averaged 21.2 t ha^{-1} of TDM, similar to those reported in a separate study near Boone, IA (Jarchow and Liebman, 2012).

3.6. Harvest index and nitrogen harvest index

Both the HI and NHI were significantly influenced by N treatment (Table 5). However, no interaction between N treatment and environment was observed. The treatments T2, T4, T5, T7 and T2, T4, T6, T7 had greater HI and NHI, respectively than the other four N treatments. These significant different among N treatments indicates that the allocation of N to grain was higher in some of the alternative N treatments compared to the control treatments (T1 and T8). The LYS byproduct and TRP byproduct provided 75% and 25% of the total N rate, respectively in treatment T7. Mean HI and NHI values for this treatment were 0.52 and 0.67 kg kg^{-1}, respectively, being the greatest values among N treatments. The HI and NHI mean values reported in this study are in agreement with those reported by Ciampitti and Vyn (2012), in a comprehensive review of various corn physiological aspects.

Others studies conducted under control conditions, greenhouses, have reported positive effects on different crop plants due to physiological effects (e.g., auxin production) rather than nutritional factors in soils following TRP applications (Frankenberger et al., 1990; Frankenberger and Arshad, 1991; Arshad et al., 1995). We believe that the observed improvement of the HI and NHI indices when N supply came partially or totally from biosynthesis byproducts was likely due to effects of amino acids or their transformed secondary metabolites (e.g., plants growth regulators) on internal physiological mechanisms driving N allocation in corn. These findings may lead to further investigation to elucidate the physiological factors underlying these variations in N utilization in response to N from amino acids sources.

3.7. In-Season plant measurements

The LAI, NDVI and SPAD varied considerably among environments. But the N treatment × environment interaction factors was significant only for LAI and NDVI measurements, therefore environments were analyzed separately (Figures 2 and 3).

As the growing season progressed, the LAI, NDVI and SPAD parameters increased gradually for all environments regardless of N treatments. The last measurements during the 2013 and 2014 growing seasons were taken when corn was at R3 and R2 reproductive corn stages, respectively. On 23 July 2013, when corn plots were at or close to V18 growth stage a hail storm occurred at the experimental site, and it likely this hail storm negatively affected corn growth and yield during the 2013 growing season.

3.7.1. Leaf area index

Corn LAI was significantly influenced by date in all environments (Table 6). However, LAI was significantly influenced by N treatments only at SF I ($P < 0.05$) (Table 6 and Fig. 2). At SF I, and across N treatments and measurement dates, LAI averaged 3.6 units, while at AF the LAI was 4.8, representing the lowest and highest mean values, respectively, in this study. The LAI across measurement dates and N treatments ranged from 0.94 to 7.68 across environments. In general during the period of measurements maximum LAI values were observed around VT growth stage, following by a slight decline during reproductive stages (Fig. 2.).

At SF I, from V8 (first measurement date) on, LAI values showed consistent relative difference among N treatments during the measurement period, in the order T1 = T2 = T3 = T5

= T8 > T4 ≈ T6 ≈ T7. The T8 treatment (AS) averaged 4.4 units across measurements and consistently had the highest LAI. In contrast, the T6 (N rate from 50% TRP byproduct +50% LYS byproduct) plots had the lowest LAI average, 2.8 (Fig. 2A)

At SF II, a significant (*P* < 0.001) N treatment × measurement date interaction was observed on LAI (Table 6). The LAI values were inconsistent among N treatments. This interaction effect appeared soon after the consecutive measurements were commenced at this environment (V10) and continued until the mid-season measurement dates (around VT) and disappeared at late measurement dates. The T1, T4, T5, T6 and T7 N treatments had greater leaf area compared to the other N treatments on these mid-season measurement dates. The interaction effect between N treatments and measurement date was not significant on later dates (R1 to– R3) (Fig. 2B).

At AF, neither N treatment nor the N treatment × date interaction was significant (Table 6 and Figure 2C). Averaged across N treatments, mean LAI was 4.82 (results not presented).

3.7.2. Normalized difference vegetation index

The N treatment main effect was not significant for NDVI in any of the three environments, but measurement dates were significant for NDVI in each environment. However, the response to N treatments was inconsistent among environments (Table 6 and Fig. 3) as indicated by the two way interaction of N treatment × measurement date. The NDVI through the two growing seasons and across N treatments ranged from 0.59 to 0.74 at SF I, 0.67 to 0.79 at SF II and 0.76 to 0.81 at AF. Similar ranges of NDVI values were shown by Martin et al. (2007).

At SF I, significant interactions between sampling date and N treatment were detected along the seven consecutive measurements dates of this study. These interactions occurred during mid-vegetative sampling dates (V8 –V14), where the T1, T8 and T2 N treatments had higher NDVI values than the rest of the eight N treatments. The NDVI values slightly decreased during the third (~V 13) to the last measurement date (R3) (Fig. 3A).

At SF II, NDVI values showed an increase between the two initial measurement dates (V8 – V11). Subsequently, the values for each N treatment remained relatively constant until the last measurement date (R2). No main or interaction effects were observed at this environment (Table 6 and Fig. 3B).At AF, there were only small changes in the NDVI values between the V8 growth stage and the R3 growth stage and the seasonal NDVI pattern at this research site was similar to the previously described for SF II (Fig. 3C).

In spite of differences found in the measurements of light reflectance from the corn canopy at one study environment (SF I) during mid-season measurements; no differences in NDVI were found at late-season measurements at any study environments. Being that NDVI sensing values are an indicator of plant growth, it has been used as a predictor of both corn grain and biomass yields (Elwadie et al., 2005; Martin et al., 2007; Yin and McClure, 2013). Our results suggest that late-in-season NDVI values were well related to the no differences found in terms of corn grain yield production among N treatments, but they were not well related to the differences found in biomass yields. Nonetheless, at SF I two out of the three treatments with the greatest NDVI values at mid-season sampling dates (T1 and T8), concomitantly were the ones with greatest corn biomass productivity across environments.

3.7.3. Leaf greenness

Across environments, SPAD readings did not differ by N treatment or the N treatment × environment interaction; thus SPAD data across N treatments were pooled at each study environment. The SPAD readings were highly influenced by measurement date at all environments ($P < 0.001$) (results not presented). As indicated by the lack of N treatment x environment interaction, the response to N was similar across environments. The mean SPAD readings varied from 28.8 to 64.6, which is typical for corn (Ziadi et al., 2008; Scharf et al., 2006). Across N treatments and measurement dates at SF II averaged SPAD (53.5) was greater in comparison to the other two study environments. In 2013, the sharp decline in SPAD during week fourth of in-season measurements was due to the hail storm damage.

3.8. Nitrogen uptake and concentration

Grain N concentration differed among N treatments ($P < 0.05$), but the N treatments did not significantly affect N concentrations in corn stover and cobs. Moreover, grain, stover and cobs N concentration varied among environments, but N treatment x environment interactions were not significant indicating that the N treatment responses were consistent across environments. Averaged across N treatments for all environments, SF I had the lowest grain (11.2 g kg^{-1}), stover (6.4 g kg^{-1}) and cob (1.6 g kg^{-1}) mean N concentrations (results not presented). When averaged across study environments the T1, T3, T6 and T7 treatments had the highest grain N concentrations (Table 5).

Stover N uptake was influenced by N treatments ($P < 0.05$); although both corn grain and cob N uptake were not influenced by N treatments across environments (Table 5). Similarly to most of the productive variables the N uptake of the three corn plant components differed among environments (Table 5).

Corn stover N uptake was greater in T1, T3 and T8. Grain N uptake varied from 77 to 138 kg ha^{-1}, 107 to 151 kg ha^{-1} and 108 to 151 kg ha^{-1} at SF I, SF II and AF respectively. Cob N uptake varied from 0 to 5 kg ha^{-1}, 6 to 11 kg ha^{-1} and 4 to 9 kg ha^{-1} at SF I, SF II and AF respectively. Stover N uptake varied from 23 to 69 kg ha^{-1}, 7 to 141 kg ha^{-1} and 50 to 108 kg ha^{-1} at SF I, SF II and AF, respectively. These results are similar to those reported by Sindelar et al. (2013), where stover and cob N uptake increased as N fertilizers rates increased in a N rate study in Minnesota.

Among environments, corn cob N uptake (7.8 kg ha^{-1}) and grain N uptake (128.5kg ha^{-1}) were higher at SF II compared to SF I and AF, while stover N uptake (80.72 kg ha^{-1}) at AF was the greatest among the environments considered in this study (results not presented). As previously noted only two (grain N concentration and stover N uptake) out seven parameters that described N concentration and N uptake in this study were influenced by N treatments. For control treatments (T1 and T8) the higher stover N uptake was related with higher biomass production; however, this was not the case for T3 that had a stover N uptake as high as for the control treatments but its corn stover production was lower than the control treatments. A higher grain N concentration for T1, T3, T6 and T7 was not related with higher grain production in these treatments in comparison to the remaining N treatments. However, the T6 and T7 treatments were the ones that also exhibited enhanced grain N partitioning.

3.9. Grain quality

Corn grain protein and oil concentration were similar among N treatments and averaged 70.4 and 37.5 g kg^{-1}, respectively. These protein and oil levels are similar to those reported by Miao et al. (2006) in a N rate study conducted in Illinois. Also, no N treatment × environment interaction occurred for oil and protein concentration. However, both grain quality parameters differed among environments (Table 5). Oil (36.9 g kg^{-1}) and protein (61.7 g kg^{-1}) concentration were the lowest at SF I, while AF and SF II exhibited the highest oil (38.0 g kg^{-1}) and protein (75.6 g kg^{-1}) concentration values, respectively (results not presented).

Considerable attention is currently being given to environmental implications of N sources in agriculture production. Both TRP and LYS are hydrophobic and polar charged amino acids, respectively, a trait that makes the N in these amino acids less susceptible to losses by leaching. Future research could also explore the N dynamics in soils when N is applied in the form of amino acids to investigate how N loading to water systems is affected by using these alternative N sources. Also, an accurate estimation of N mineralization rate would provide more insights on the amount of potentially mineralizable N and the rate at which N mineralization occurs when amino acids and their byproducts are utilized in corn production. Such a study will allow making more comprehensive conclusions for practical purposes.

4. Conclusions

Our field study demonstrated that amino acids and their biosynthesis byproducts are a suitable source of N for corn production. Soil applications of these alternative N sources provided similar corn yields as commonly used synthetic N fertilizers sources, AN and AS. Likewise, utilization of TRP and LYS byproducts did not affect corn growth and development as demonstrated by no differences among N treatments at late in the season measurement dates of LAI, NDVI and SPAD values. However, further research is needed in order to identify potential impacts associated with long term use of biosynthesis byproducts on soil and environmental parameters.

Amino acid biosynthesis byproducts can replace synthetic AN and AS to produce high corn yields. However, corn stover production was decreased by the use of any of the alternative N treatments, except when TRP and LYS byproducts were combined to provide 98 kg N ha^{-1} each. The ability to sustain good corn grain yields is very likely due to their high nutrient availability as indicated by the chemical compositions of the alternative N sources. Our results also highlight that byproducts utilization enhance N grain partitioning relative to AN and AS fertilizers. We believe that this finding may be related to physiological alterations in the partitioning and dynamics due to the presence of plant growth regulators when N is provided from amino acid sources such as TRP. Further investigation of these N partitioning patterns in corn fertilized with N from amino acids will further improve our understanding of this dynamic.

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References

Ahmad, R., Khalid, A. H., Arshad, M., Zahir, A., Mahmood, T., 2007. Effect of compost enriched with N and L-tryptophan on soil and maize. Agron. Sustain. Dev. 28, 1-7.

Ajinomoto Inc. 2004, FY 2003 market and other information. Retrieved from: https://www.ajinomoto.com/en/ir/pdf/q1_fy2004_data.pdf

Ajinomoto Inc. 2015, FY 2014 market and other information. Retrieved from: http://www.ajinomoto.com/en/ir/pdf/FY14_Data_E.pdf

Arshad, M., Hussain, A., Shakoor, A., 1995. Effect of soil applied L-tryptophan on growth and chemical composition of cotton. J. Plant Nutr. 18, 317-329.

Bitzer, C.C., Sims, J. T., 1988. Estimating the availability of nitrogen in poultry manure through laboratory and field studies. J. Environ. Qual. 17, 47–54.

Chadwik, D. R., John, F., Pain, B. F., Chamber, B. J., Williams, J., 2000. Plant uptake of nitrogen from the organic nitrogen fraction of animal manures: a laboratory experiment. J. Agr. Sci. 134, 159-168.

Chapin, E S., Moilanen, L., Kielland, K., 1993. Preferential usage of organic nitrogen for growth by a non-mycorrhizal sedge. Nature 361, 150-152.

Cassman, K.G., Peng, S., Olk, D. D., Ladha, J. K., Reichardt, w., Dobermann, A., Singh. U., 1998. Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. Field Crops Res. 56, 7–39.

Cassman, K.G., Dobermann, A., Walters, D., 2002. Agroecosystems, nitrogen-use efficiency, and nitrogen management. AMBIO 31, 132-140.

Ciampitti, I.A., Vyn, T. J., 2012. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. Field Crops Res. 133, 48–67.

Dobermann, A., 2005. Procedure for Measuring Dry matter, Nutrient uptake, Yield and Components of Yield in Maize. Retrieved from: global maize project: http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB8QFjAA&url=http%3A%2F%2Fwww.globalmaize.org%2Fmedia%2Ffiles%2F1_07oct05_seap_61.doc&ei=jxuLVb6yE4GuyATNjYCQCg&usg=AFQjCNE9QXJw8W2P0cJRAgNB4Rs1Mk7ogg

Duvick, D.N., 2005. The contribution of breeding to yield advances in maize (*Zea mays* L.). Adv. Agron. 86, 83–145.

Economic Research Service. 2011. Fertilizer use and price. Retrieved from: Economic Research Service: http://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx

Edwards, DR., Daniel, T. C., 1992. Environmental impact of on-farm poultry waste disposal a review. Bioresource Technol. 41, 9-33.

El-Awadi, M. E., El-Bassiony, A. M., Fawzy, Z. F., El-Nemr, M. A., 2011. Response of snap bean (*Phaseolus vulgaris* L) plants to nitrogen fertilizer and foliar application with methionine and tryptophan. J. Nat. Sci. 9, 87-94.

Elwadie, M.E., Pierce, F. J., Qi, J., 2005. Remote sensing of canopy dynamics and biophysical variables estimation of corn in Michigan. Agron. J. 97, 99–105.

Frankenberger, W. T. Jr., Chang A. C., Arshad, M., 1990. Response of *Raphanus sativus* to the auxin precursor L-tryptophan applied to soil. Plant Soil 129, 235-241.

Frankerbeger, W. T. Jr., Arshad, M., 1991. Yield response of *Capsicum Annuum* to the auxin precursor, L-tryptophan applied to soil. PGRSA Quart. 19, 231-240.

Frankenberger, W. T. Jr., Arshad, M., 1991b. Yield response of watermelon and muskmelon to L-tryptophan applied to soil. Hort. Sci. 26, 35-37.

Franzen, D.W., and J. Lukach. 2007. Fertilizing canola and mustard. Retrieved from: North Dakota St. Univ. Extension: https://www.ag.ndsu.edu/pubs/plantsci/soilfert/sf1122.pdf

Grassini, P., Specht, J. E., Tollenaar, M., Ciampitti, I., Cassman, K. G., 2015. High-yield maize-soybean cropping systems in the US corn belt, in: Sadras, V.O., Calderini, D.F. (Eds), Crop physiology- applications for genetic improvement and agronomy (2nd edition), Elsevier, The Netherlands, pp 17-41.

Gitelson, A.A., Kaufman, Y. J., Merzlyak, M. N., 1996. Use of a green channel in remote sensing of global vegetation from EOS-MODIS. Remote Sens. Environ. 58, 289–298.

Havlin, J.L., Tisdale, S. L., Beaton, J. D., Nelson, W. L., 2005. Soil fertility and fertilizers. Pearson Education, Inc., Upper Saddle River, NJ

Henning, S., 2007. Corn and soil test response to by-Product nitrogen sources. Iowa State Research Farm Progress Reports. Paper 733.

International fertilizer industry association. 2015. World nitrogen fertilizer consumption. Retrieved from: International fertilizer industry association: http://www.fertilizer.org/En/Knowledge_Resources/About_Fertilizers/Main_Fertilizer_Products.aspx?WebsiteKey=411e9724-4bda-422f-abfc-8152ed74f306

Jarchow, M.E., Liebman, M., 2012. Tradeoffs in biomass and nutrient allocation in prairies and corn managed for bioenergy production. Crop Sci. 52, 1330–1342.

Johnson, J. M. –F., Allmaras, R. R., Reicosky, D. C., 2006. Estimating source of carbon from crop residues, roots and rhizodeposits using the national grain yield-data base. Agron. J. 98, 622-636.

Karlen, D.L., Birell, S.J., Hess, J. R., 2011. A five-year assessment of corn stover harvest in central Iowa, USA. Soil Tillage Res. 116, 47–55.

Leikam, D.L., Lamond, R.E., Mengel, D. B., 2003. Soil test interpretations and fertilizer recommendations. Retrieved from: Kansas State Univ. Res. and Ext., Manhattan, KS: http://www.agronomy.k-state.edu/services/soiltesting/fertilizer-recommendations.html

Lithourgidis, A.S., Matsi, Th., Barbayiannis, N., Dordas, C. A., 2007. Effect of liquid cattle manure on corn yield, composition, and soil properties. Agron. J. 99, 1041–1047.

Loecke, T.D., Liebman, M., Cambardella, C. A., Richard, T. L., 2004. Corn growth responses to composted and fresh solid swine manures. Crop Sci. 44, 177–184.

Mallarino, A., Sawyer, J. E., Barnhart, S. K., 2013. A general guide for crop nutrient and limestone recommendations in Iowa. Retrieved from Iowa State Univ. Extension:

https://store.extension.iastate.edu/Product/A-General-Guide-for-Crop-Nutrient-and-Limestone-Recommendations-in-Iowa

Markwell, J., Osternman, J. C., Mitchell, J.L., 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynth. Res. 46, 467–472.

Martin, K.L., Girma, K., Freeman, K. W., Teal, R. K., Tubana, B., Arnall, D. B., Chung, B., Walsh, O., Solie, J. B., Stone, L., Raun, W. R., 2007. Expression of variability in corn as influenced by growth stage using optical sensor measurements. Agron. J. 99, 384–389.

Martinez, C. E., Tabatabai, M. A., 1997. Decomposition of biotechnology by-products in soils. J. Environ. Qual. 26, 625-632.

Mathur, S.P., Owen, G., Dinel, h., Schnitzer, M., 1993. Determination of compost biomaturity. I. Literature review. Biol. Agric. Hortic. 10, 65–85.

Miao, Y., Mulla, D. J., Robert, P. C., Hernandez, J . A., 2006. Within-field variation in corn yield and grain quality responses to nitrogen fertilization and hybrid selection. Agron. J. 98, 129-140.

Mikkelsen, R., Hartz, T. K., 2008. Nitrogen sources for organic production. Better crops 92, 16-19.

Motavalli, P.P., Kelling, K. A., Converse, J.C., 1989. First-year nutrient availability from injected dairy manure. J. Environ. Qual. 18, 180–185.

National Agricultural Statistics Service. 2011. Acreage. Retrieved from: USDA: National Agricultural Statistics Service: http://www.nass.usda.gov/Newsroom/2013/06_28_2013.asp

Näsholm, T., Huss-Danell, K., Högberg, P., 2000. Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81, 1155-1161.

Owen, A.G., Jones, D.L., 2001. Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biol. Biochem. 33, 651-657.

Öhlund, J., Näsholm, J., 2004. Regulation of organic and inorganic nitrogen uptake in scots pine (*Pinus sylvestris*) seedlings. Tree Physiol. 24, 1397-1402.

Peoples, M.B., Herridge, D.F., Ladha, J. K., 1995. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? Plant Soil 174, 3–28

Peterson, T.A., Blackmer, T. M., Francis, D. D., Schepers, J. S., 1993. Using a chlorophyll meter to improve N management. Retrieved from: Univ. of Nebraska, Lincoln extension: http://ianrpubs.unl.edu/live/g1632/build/g1632.pdf

Ruiz Diaz, D.A., Sawyer, J. E., 2008. Plant-Available nitrogen from poultry manure as affected by time of application. Agron. J. 100, 1318-1326.

Ruiz Diaz, D.A., Sawyer, J. E., Barker, D. W., 2012. Residual poultry manure nitrogen supply to corn the second and third years after application. Soil Sci. Soc. Am. J. 76, 2289-2296.

Sarwar, M., Frankenberger, W. T. Jr., 1994. Influence of L-tryptophan and auxins applied to the rhizosphere on the vegetative growth of Zea mays L. Plant Soil 160, 97-104.

Sawyer, J. E. 2012. Soil Nitrogen Cycle. Iowa State University. Presentation at the Climate and corn-based cropping systems coordinated agricultural project (CSCAP) 2012 Annual Meeting.

Sawyer, J.E., Lang, B., Barker, D. W., Cummins, G., 2009. Dealing with sulfur deficiency in crop production: the Iowa experience. p. 64-73. In Proc. Thirty-Ninth North Central Extension-Industry Soil Fertility Conf., Des Moines, IA. 18-19 Nov. 2009. Vol. 25. International Plant Nutrition Inst., Brookings, SD.

Scharf, P.C., Brouder, S. M., Hoeft, R. G., 2006. Chlorophyll meter readings can predict nitrogen need and yield response of corn in the north-central USA. Agron. J. 98, 655–665.

Schobert, C., Köckenberger, W., Komor, E., 1988. Uptake of amino acids by plants from the soil: A comparative study with castor bean seedlings grown under natural and axenic soil conditions. Plant Soil 109, 181-188.

Schepers, J.S., Francis, D. D., Vigil, M., Below, F. E., 1992. Comparison of corn leaf nitrogen and chlorophyll meter readings. Commun. Soil Sci. Plant Anal. 23, 2173–2187.

Sindelar, A. J., Coulter, J. A., Lamb, J. A., Vetsch, J. A., 2015. Nitrogen, stover and tillage management affect nitrogen use efficiency in continuous corn. Agron. J. 107, 843-850.

Sindelar, A. J., Lamb, J. A., Sheaffer, C., Rosen, C. J., Jung, H. G., 2013. Fertilizer nitrogen rate effects on nutrient removal by corn stover and cobs. Agron. J. 105, 437-445.

Singer, J. W., Moore, K. J., Hintz, R. L., Patrick, P. K., 2007. Effect of source and rate of nitrogen and sulfur fertilizer on yield, quality and mineral composition of stockpiled tall fescue. Crop Manage. 6, 1-10.

Tilman, D., 1998. The greening of the green revolution. Nature 396, 211-212.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671-677.

U.S. Canola Association, 2015. Crop Production. Retrieved from: U.S. Canola Association: http://www.uscanola.com/crop-production/

Villamil, M. B., Little, J., Nafizger, E. D., 2015. Corn residue, tillage, and nitrogen rate effects on soil properties. Soil Tillage Res. 151, 61–66.

Warren, C. R., 2006. Potential organic and inorganic N uptake by six Eucalyptus species. Funct. Plant Biol. 33, 653-660.

Warren, C. R., Adams, P. R., 2006. Uptake of nitrate, ammonium and glycine by plants of Tasmania wet eucalypt forests. Tree Physiol. 27, 413-419.

Wilson, D.M., Heaton, E. A., Liebman, M., Moore, K. J., 2013. Intraseasonal changes in switchgrass nitrogen distribution compared with corn. Agron. J. 105, 285–294.

Woli, K. P., Rakshit, S., Lundvall, J. P., Sawyer, J. E., Barker, D. W., 2012. On-farm evaluation of liquid swine manure as a nitrogen source for corn production. Agron. J. 105, 248-262.

Yin, X., McClure, M. A., 2013. Relationship of corn yield, biomass, and leaf nitrogen with normalized difference vegetation index and plant height. Agron. J. 105, 1005-1016.

Zhu, B., Tabatabai, M.A., Henning, S. J., 1995. Biotechnology by-products as sources of nitrogen for plants. J. Environ. Qual. 24, 986-992.

Ziadi, N.,Brassard, M., Bélanger, G., Claessens, A., Tremblay, N., Cambouris, A. N., Nolin, M. C., Parent., L. E., 2008. Chlorophyll measurements and nitrogen nutrition index for the evaluation of corn nitrogen status. Agron. J. 100, 1264–1273.

Table 1. Nitrogen component sources for eight isonitrogenous fertilizer treatments applied to corn in three environments in Iowa, 2013- 2014. The values are the kg N ha-1 provided by each N source.

Treatment[a]	Formulation	TRP[b]	TRP Byproduct	LYS Byproduct[c]	Ammonium Nitrate	Ammonium Sulfate
		kg N ha^{-1}				
T1	Dry	0	0	0	196	0
T2	Dry	14	0	0	182	0
T3	Dry	26	0	0	170	0
T4	Dry	56	0	0	140	0
T5	Liquid	0	196	0	0	0
T6	Liquid	0	98	98	0	0
T7	Liquid	0	48	148	0	0
T8	Dry	0	0	0	0	196

[a] T1= ammonium nitrate (AN) 196 kg N ha^{-1}; T2= pure tryptophan (TRP) 14 kg N ha^{-1} + AN 182 kg N ha^{-1}; T3= pure TRP 26 kg N ha^{-1} + AN 170 kg N ha^{-1}; T4= pure TRP 56 kg N ha^{-1} + AN 140 kg N ha^{-1}; T5= TRP byproduct 196 kg N ha^{-1}; T6= TRP byproduct 98 kg N ha^{-1} + Lysine byproduct 98 kg N ha^{-1}; T7= TRP byproduct 48 kg N ha^{-1} + Lysine byproduct 148 kg N ha^{-1}; T8= ammonium sulfate 196 kg N ha^{-1}.

[b] TRP= tryptophan

[c] LYS= lysine

Table 2. Soil properties for 0- to 15-cm depth collected within one week prior corn planting.

Soil parameter	SF I[a]	SF II[b]	AF[c]
Organic matter, g kg^{-1}	33	26	31
NO_3-N, mg kg^{-1}	8	7	13
Mehlich-3 P, mg kg^{-1}	12	10	45
Mehlich-3 K, mg kg^{-1}	96	97	195
pH	6.50	5.55	6.45

[a] SF= Sorenson farm research environment 2013

[b] SF= Sorenson farm research environment 2014

[c] AF= Agronomy farm research environment 2014

Table 3. Chemical composition of the amino acids and amino acids byproducts applied in 2013 and 2014.

Product and byproducts parameters	TRP[a]	TRP Byproduct	LYS Byproduct[b]
Total N, g kg^{-1}	136	5.4	65
NH_4^+-N, g kg^{-1}	2.4	1.1	53.6
NO_3^--N, g kg^{-1}	<0.01	<0.01	<0.01
Organic N, g kg^{-1}	134	5	15
Total P, g kg^{-1}	<0.01	0.9	2.8
Total K, g kg^{-1}	0.1	1.7	6.6
S, mg kg^{-1}	173	4105	74900
Ca, mg kg^{-1}	41	42	223
Mg, mg kg^{-1}	3	45	322
Fe, mg kg^{-1}	1.3	0.5	15.6
Zn, mg kg^{-1}	1.4	0.5	1.7
Cu, mg kg^{-1}	0.50	<0.01	0.03
pH	4.0	3.0	3.5
C:N ratio	4.7	3.8	1.3

[a] TRP= tryptophan

[b] LYS= lysine

Table 4. Amounts of N sources added and total N, P, K and S applied to each N treatment at experimental plots in Ames, IA 2013 and 2014.

Treatment[a]	Source					Total			
	AN[b]	AS[c]	TRP[d]	TRP byproduct	LYS byproduct[e]	N	P	K	S
	kg ha^{-1}			L ha^{-1}		kg ha^{-1}			
T1	576	0	0	0	0	196	0	0	0
T2	535	0	103	0	0	196	0	0	0
T3	500	0	191	0	0	196	0	0	0
T4	412	0	412	0	0	196	0	0	0
T5	0	0	0	36395	0	196	33	62	149
T6	0	0	0	18197	1512	196	21	41	188
T7	0	0	0	9099	2268	196	15	30	207
T8	0	933	0	0	0	196	0	0	224

[a] T1= ammonium nitrate (AN) 196 kg N ha^{-1}; T2= pure tryptophan (TRP) 14 kg N ha^{-1} + AN 182 kg N ha^{-1}; T3= pure TRP 26 kg N ha^{-1} + AN 170 kg N ha^{-1}; T4= pure TRP 56 kg N ha^{-1} + AN 140 kg N ha^{-1}; T5= TRP byproduct 196 kg N ha^{-1}; T6= TRP byproduct 98 kg N ha^{-1} + Lysine byproduct 98 kg N ha^{-1}; T7= TRP byproduct 48 kg N ha^{-1} + Lysine byproduct 148 kg N ha^{-1}; T8= ammonium sulfate 196 kg N ha^{-1}.

[b] AN= ammonium nitrate

[c] AS= ammonium sulfate

[d] TRP= pure tryptophan

[e] LYS= lysine

Table 5. The effect of environment (E) and N treatment (T) across 2 yrs. on corn grain yield, corn stover yield, corn total aboveground biomass (TAB), harvest index (HI), grain nitrogen concentration, stover nitrogen concentration, cobs nitrogen concentration, grain nitrogen uptake, stover nitrogen uptake, cobs nitrogen uptake, total aboveground nitrogen uptake (TNU), nitrogen harvest index, grain protein (GP) concentration and grain oil (GO) concentration at Ames, IA, 2013 and 2014. Grain yield is expressed for 155 g kg^{-1} moisture content whereas other yield variables are on an oven-dry basis.

N treatment[a]	Grain yield	Stover yield	TAB	HI	Grain N	Stover N	Cob N	Grain N	Stover N	Cob N	TNU	NHI	GP	GO
	t ha^{-1}			kg kg^{-1}	g kg^{-1}			kg ha^{-1}				kg kg^{-1}	g kg^{-1}	
T1	12.5	9.7a	22.1	0.50d	11.8ab	8.4	3.4	129.5	81.8a	5.3	216	0.61b	70.6	37.4
T2	12.3	8.6b	20.8	0.52ab	11.5bc	7.6	3.6	123.8	65.9bc	5.1	195	0.64ab	69.7	37.1
T3	11.6	8.6b	20.1	0.50cd	12.0a	8.4	3.7	120.6	73.4abc	5.3	199	0.61b	72.1	36.8
T4	11.7	8.6b	20.2	0.51abcd	11.6bc	7.1	3.7	118.8	62.9c	5.6	186	0.65ab	69.3	37.1
T5	12.1	8.5b	20.5	0.52abc	11.2c	7.6	3.8	118.5	64.7bc	5.6	188	0.63b	67.3	38.2
T6	11.9	9.0ab	20.8	0.50bcd	11.7ab	6.9	4.2	122.9	62.7c	6.1	190	0.65ab	70.3	37.7
T7	12.4	8.5b	20.8	0.52a	11.6abc	6.8	4.0	126.9	58.5c	6.2	190	0.67a	70.0	37.6
T8	12.9	9.3a	22.4	0.50bcd	11.5bc	8.2	3.5	129.8	80.1ab	5.3	215	0.61b	68.4	37.4
SE	0.51	0.45	0.88	0.01	0.21	0.76	0.31	4.95	7.87	0.52	11.56	0.02	14.3	0.56
Significance							*P*-value							
Environment (E)	NS[c]	**	*	***	**	**	***	*	***	***	***	***	***	**
Treatment (T)	NS	*	NS	*	*	NS	NS	NS	*	NS	NS	*	NS	NS
E × T	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] T1= ammonium nitrate (AN) 196 kg N ha^{-1}; T2= pure tryptophan (TRP) 14 kg N ha^{-1} + AN 182 kg N ha^{-1}; T3= pure TRP 26 kg N ha^{-1} + AN 170 kg N ha^{-1}; T4= pure TRP 56 kg N ha^{-1} + AN 140 kg N ha^{-1}; T5= TRP byproduct 196 kg N ha^{-1}; T6= TRP byproduct 98 kg N ha^{-1} + Lysine byproduct 98 kg N ha^{-1}; T7= TRP byproduct 48 kg N ha^{-1} + Lysine byproduct 148 kg N ha^{-1}; T8= ammonium sulfate 196 kg N ha^{-1}.

[b] Means followed by different lowercase letter within a column are significantly different P ≤ 0.05 by the least square means test.

[c] Not significant; *= Significant at P ≤ 0.05; **= Significant at P ≤ 0.01; ***= Significant at P ≤ 0.001.

Table 6. Analysis of variance of Leaf Area Index (LAI) and normalized difference vegetative index (NDVI) at the three study environments in central IA, as affected by N treatments, measurement date and the interaction of these two factors.

Environment	Effect	LAI	NDVI
Sorenson Farm 2013	N Treatments (N)	*[a]	NS
	Measurement date (D)	***	***
	N × D	NS	**
Sorenson Farm 2014	N Treatments	NS	NS
	Measurement date	***	***
	N × D	***	NS
Agronomy Farm 2014	N Treatments	NS	NS
	Measurement date	***	***
	N × D	NS	NS

[a] ns= not significant (P > 0.05); *= Significant at *P* ≤ 0.05; **= Significant at *P* ≤ 0.01; ***= Significant at *P* ≤ 0.001

Fig. 1. Monthly average precipitation (A) and monthly average temperature (B). Both climate parameters (bars) were collected from an automated weather station located < 5 km from research area in Boone, IA. Average 20-yr (line) was obtained from the Iowa Mesonet (2014).

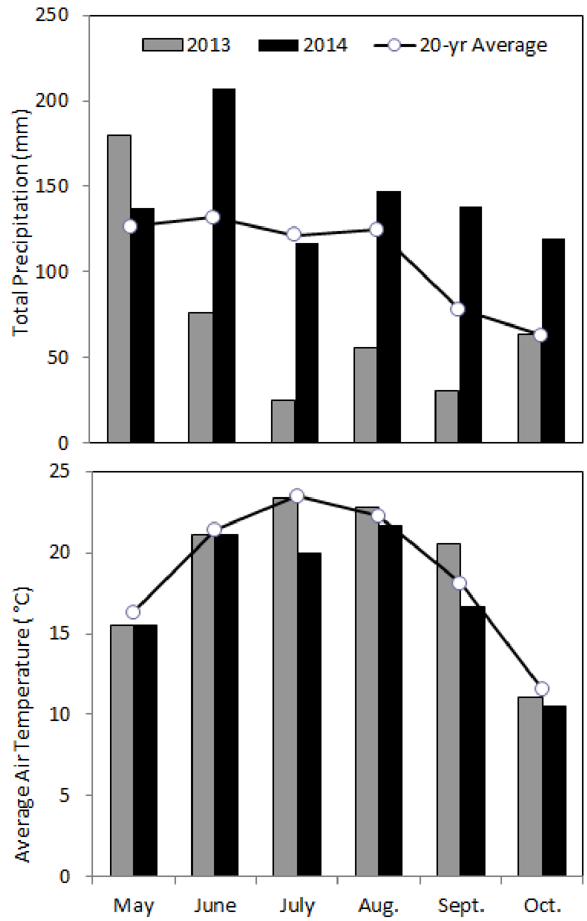
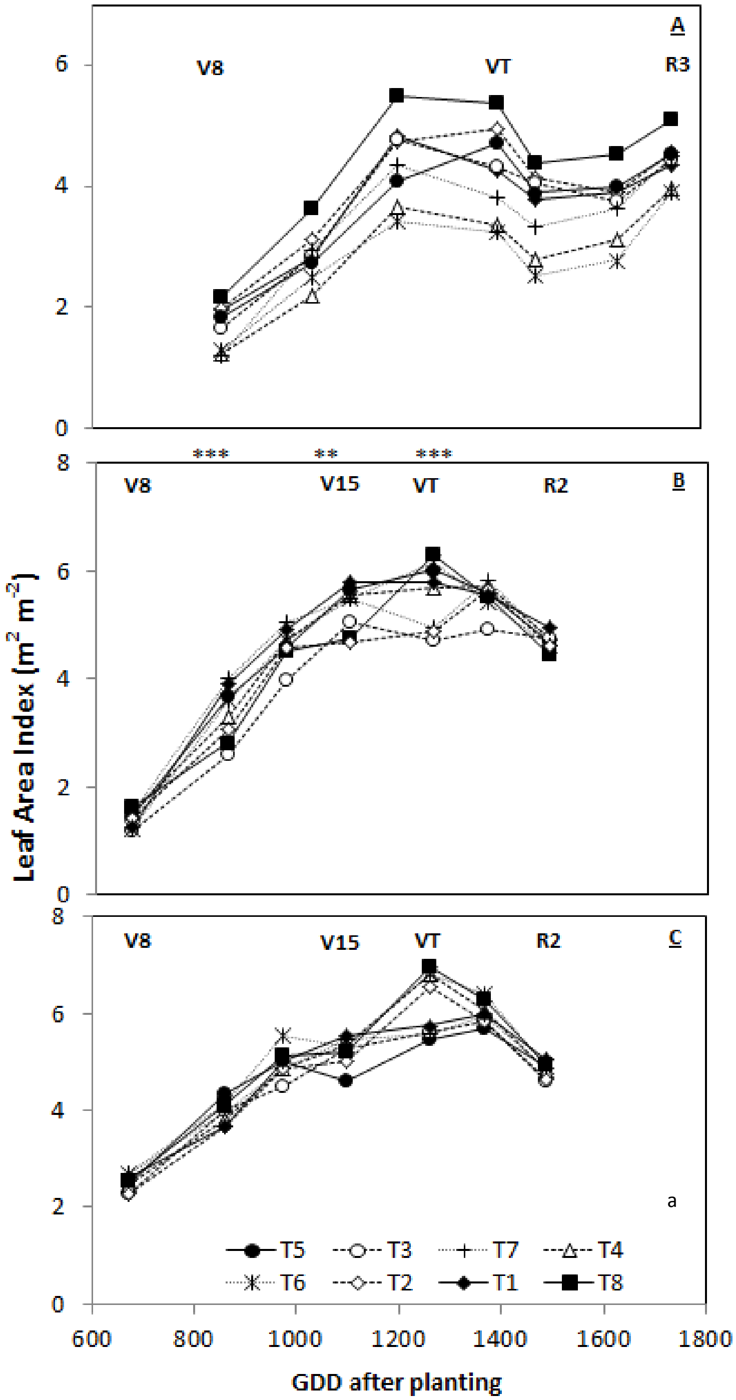


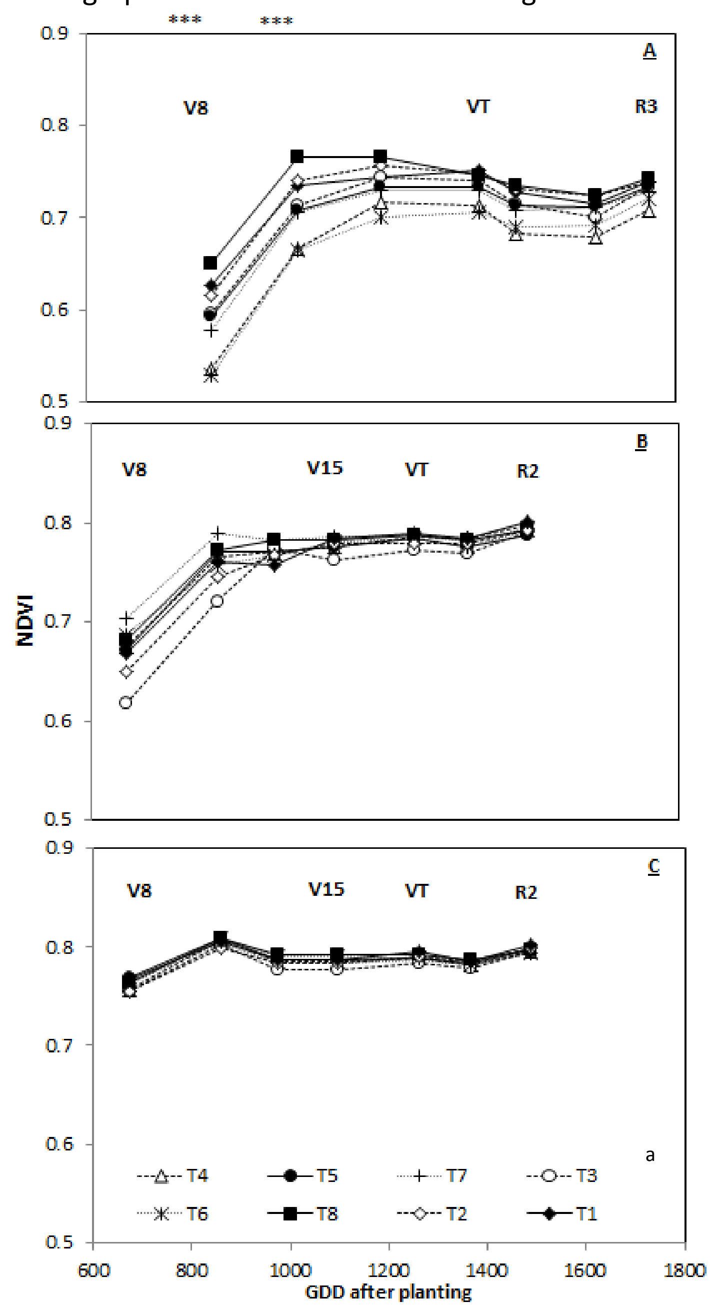
Fig. 2. Leaf area index (LAI) as a function of N treatments, during corn growth stages ranging from V8 to R3. At (A) Sorenson farm site in 2013; (B) Sorenson farm site in 2014 and (C) Agronomy farm site in 2014 near Ames, IA. Asterisks indicate significant differences for a sampling date among N treatments; if no asterisk is shown above each site graph then the difference is not significant.



[a] T1= ammonium nitrate (AN) 196 kg N ha^{-1}; T2= pure Tryptophan (TRP) 14 kg N ha^{-1} + AN 182 kg N ha^{-1}; T3= pure TRP 26 kg N ha^{-1} + AN 170 kg N ha^{-1}; T4= pure TRP 56 kg N ha-

^{1} + AN 140 kg N ha^{-1}; T5= TRP byproduct 196 kg N ha^{-1}; T6= TRP byproduct 98 kg N ha^{-1} + Lysine byproduct 98 kg N ha^{-1}; T7= TRP byproduct 48 kg N ha^{-1} + Lysine byproduct 148 kg N ha^{-1}; T8= ammonium sulfate 196 kg N ha^{-1}.

Fig. 3. Normal difference vegetative index (NDVI) as a function of N treatments, during corn growth stages ranging from V8 to R3. At (A) Sorenson farm site in 2013; (B) Sorenson farm site in 2014 and (C) Agronomy farm site in 2014 near Ames, IA. Asterisks indicate significant differences for a sampling date among N treatments; if no asterisk is shown above each site graph then the difference is not significant.



[a] T1= ammonium nitrate (AN) 196 kg N ha^{-1}; T2= pure Tryptophan (TRP) 14 kg N ha^{-1} + AN 182 kg N ha^{-1}; T3= pure TRP 26 kg N ha^{-1} + AN 170 kg N ha^{-1}; T4= pure TRP 56 kg N ha^{-1} + AN 140 kg N ha^{-1}; T5= TRP byproduct 196 kg N ha^{-1}; T6= TRP byproduct 98 kg N ha^{-1} +

Lysine byproduct 98 kg N ha^{-1}; T7= TRP byproduct 48 kg N ha^{-1} + Lysine byproduct 148 kg N ha^{-1}; T8= ammonium sulfate 196 kg N ha^{-1}.