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Testing N efficient, high methionine corn hybrids with organic farmers[1].

Report by Walter Goldstein

Abstract: Corn is North America's most productive and most grown cereal. But it has pollution problems due to the necessity for large amounts of nitrogen fertilizers and problems with a lack of nutritional density in its grain. This research project focused on testing new hybrids bred at the Mandaamin Institute which have potential for resolving both of these problems. These hybrids possess microbial partnerships that enable the corn to efficiently take up minerals and nitrogen without the use of fertilizers. The hybrids were studied in the context of different farming systems and soil fertility conditions. Studies with microscopy, field trials on different farms, and mineral and natural isotope analyses showed that 1) the plants exercise rhizophagic cycles with seed associated bacteria, leading to nitrogen efficiency in field trials, high levels of $\delta^{15}N$ in tissues, and grain with high protein and mineral contents; 2) these partnerships result in comparable yields to manured commercial hybrids where no manure is added to the Mandaamin hybrids; 3) Hybrids with the Mandaamin inbred C4-6 as a parent particularly express these traits and also appear to fix N_2. 4) The C4-6 based Mandaamin hybrids also respond negatively to fresh manure but positively to high organic N and to high soil protein levels resulting from cattle manure. 5) The negative effect of fresh manure applications on the C4-6 based hybrids extends to yield and mineral uptake and this problem is worse on soils with low organic matter content. 6) The Mandaamin hybrids have grain with a high nutritional value due to their high content of the essential amino acids methionine and lysine and their enhanced mineral content and this may offset a slightly lower yield. Future research should be directed towards testing and confirming concepts developed in the course of this project while extending the testing to more farms. Breeding should be fostered that focusses on stability for both microbial assisted processes of rhizophagy and N_2 fixation

[1]This research was made possible by the cooperation and assistance of numerous people, institutions, and farms. We acknowledge their part in this work and in formulating data with great thanks. First, the work would not have been possible without the help of many organic and biodynamic farmers who participated in the thinking behind the project and the work with it on their farms. The Corn and Soil Health project, centered at the University of Illinois, with OREI funds, accomplished basic soil analyses, worked up data on soils and farms, and helped in formulating experimental designs for the farm trials. This included graduate students E. Gulkirpik, M. Toc, C. Mujjabi, M. Nunez, data coordinator Emily Marriot, and Professors: C. Ugarte, M. Wander, M. Bohn, and J. Andrade. USDA-ARS, Morris, Minnesota (A.A. Jaradat, Chris Wente, and Jane Johnson), did tissue analysis of corn for minerals and helped prepare samples for isotope analysis. Foundation Organic Seed (S. Mohr) contributed seed and advice to the project. Rutgers University (J. White., A. Lotfi, K. Kingsley, and others) contributed rhizophagy research on maize seedlings, advice on interpreting our results, and continuous inspiration based on their research findings. University of Wisconsin Extension (Mike Travis) and Pepin County Conservation (Chase Cummings) helped organize farmers and events and meetings around the issue of N_2 fixing corn and helped carry out on farm research in the NW part of Wisconsin. Wood Ends Soil Testing Lab and Cornell University assisted with extra soil quality tests. At the Mandaamin Institute, J.Karnes, V. Thomas, A. Lanser, J. Mayfield and others contributed to getting the field research done. Any errors in interpretation of results are due to the author and not to any of the people mentioned above. Finally we would like to acknowledge funding from USDA-NIFA-OREI, SARE and the Ceres Trust, without which this work could not have been accomplished.

coupled with adequate yield performance and enhanced nutritional value under variable soil conditions with reduced N inputs.

Context and objectives: Corn is the major cereal crop for North America and the most productive cereal in the world. However, conventional corn production is largely dependent on the use of mineral nitrogen fertilizers. Organic farmers often depend on bought in chicken manure. Nitrate production in the soil under corn, coupled with the use of these N fertilizers results in surplus nitrate in the soil. This nitrate is denitrified by bacteria to produce nitrous oxide, a potent greenhouse gas. The excess nitrate not used by the crop leaches from the soil and causes massive pollution of wells, lakes, rivers, and also the Dead Zone off the Gulf of Mexico. Modern hybrids have been found to have an enhanced ability to foster microbes in their rhizospheres that increase nitrification in the soil and denitrification (Favela et al., 2021).

Furthermore, the-sided focus on increased yields may have reduced nutrient density of crops and thereby affect the health of end users.

This project was about evaluating the impacts and value of new, experimental hybrids which have been developed to reduce the need for nitrogen fertilizers while increasing nutrient density. These hybrids were developed at the Mandaamin Institute, mostly under organic farming conditions that were N limited. They resulted from a field based breeding program (Goldstein et al. 2012; 2019) that, up to now, has utilized 51 growing seasons in Wisconsin, Chile, Puerto Rico, and Hawaii. The program selected corn for the needs of organic farming including for better nutritional value and nitrogen efficiency. The hybrids that resulted may have potential for resolving some of the Midwest's most intractable problems with corn and N pollution that go beyond organic farming. This possibility is due to partnerships with microbes (Goldstein, 2016).

During this project the impacts and value of the Mandaamin hybrids were evaluated using on-farm testing. Objectives included 1) testing several Mandaamin hybrids on multiple farms to determine their agronomic and quality characteristics and effects of soil and soil management; 2) examining nutrient uptake, nitrogen efficiency and nitrogen fixation in the hybrids; 3) testing whether inoculating seed with N_2 fixing bacteria would improve crop performance and N efficiency.

We used replicated trials and soil analyses to estimate yield, quality, and N efficiency of the Mandaamin hybrids on different farms. Manure trials were utilized, and bacterial inoculation was added as an extra test factor because it seemed increasing relevant to the matters at hand. In the descriptions below, these trials are referred to as the SARE manure/inoculation trials.

In the course of the project, events caused changes from the initial plan. Extensive soil and plant sampling and analyses that had been planned for 2018 proved not to be possible due to illness contracted by one of the two Mandaamin Institute technicians and illness by the principal investigator at the end of that growing season. Furthermore, additional sites and plots that had not been planned in the initial proposal became available in 2019 due to funding of an OREI project together with the

University of Illinois. This OREI project involved un-replicated strip plots comparing multiple Mandaamin hybrids with commercial checks on different farms. These experiments are referred to as the varietal trials. A decision was made together with regional SARE leadership to incorporate those extra sites, to use SARE funds to better monitor these sites with soil and tissue testing, to modify the approach to the research accordingly, and to include a conventional farm into the plan. As will be shown below, pursuance of these varietal trials with SARE funds proved to be highly rewarding from the standpoint of gaining information.

Furthermore, to compensate for the first year, efforts were carried out in 2021 that included limited trials to clarify questions raised by results in preceding years and to analyze data from the overall effort.

The data gathered by the project was substantial. More analyses of the data in this report and other data are planned to further harvest pertinent results into the form of publishable papers. So this report should be regarded by the reader as preliminary information which will probably be modified in the review process. In this report we focus on the most important findings and there are a few experiments not reported on because their results were not essential to the overall findings. Aside from that, the ample tables and graphs used to describe the results have resulted in a large report. So in order to focus the reader on the outcomes of the project, rather than burying them first under voluminous tables, we will present our summary and discussion section first, then lead into the context and background sections followed by methods, description of results, and citations.

Summary and discussion of results from the project:

1) Microscopic inspection seedlings of Mandaamin inbreds that were grown under axenic conditions at Rutgers University showed the presence of large numbers of seed-borne bacteria that were multiplying and living inside root cells. These bacteria were also visibly excreted from root hairs into the rhizosphere. Microscopic stains showed that the bacteria were surrounded by oxidative substance produced by the plants to degrade the bacteria. In addition to this, observation of the putative N_2 fixing inbred C4-6 suggested a unique relationship as bacteria living in root cells appeared imbedded in some kind of gel-like, cloudy matrix.
2) These bacteria are thought to enhance nutrient availability in the rhizosphere and provide the plant with nutrients associated with oxidized microbial biomass through rhizophagy cycles. Such cycles entail the excretion of mineral depleted bacteria from root hairs and the reabsorption of mineral enriched bacteria by young growing roots.
3) Rhizophagy results in uptake of macro- and micronutrients both from turnover of microbial bodies in plant tissues and from N_2 fixation (White et al., 2018; 2019a,b). N_2 fixation involves a specific biochemical dialogue between bacteria and plant where the microbes produce nitric oxide and ammonium as antioxidants to protect themselves from superoxide produced by the plant (Chang et al., 2019; Micci et al., 2022).
4) At question is whether hybrids developed from inbreds that are rhizophagic can yield competitively with conventional hybrids. Yields of Mandaamin hybrid 17.461 were compared with yields of the commercial check FOS8500 on 14 farm sites over two years. The 17.461 averaged 139 bu/acre and the FOS8500 averaged 144 bu/acre. Corn hybrids grown on cattle

farms yielded 38 bu/acre more than the corn grown in arable organic systems which had lower contents of soil organic N. The 17.461 yielded 28 bu/acre more than the FOS8500 in the arable system without manure which was 37% more. The 17.461 yielded 24 bu/acre less than the FOS8500 in the arable system with manure which was 15% less for the standardized comparisons. The 17.461 also yielded 5 bu/acre less than the FOS8500 in the cattle system without manure which was 4% less for the standardized comparisons. The 17.461 yielded 16 bu/acre less than the FOS8500 in the cattle system with manure which was 8% less for the standardized comparisons. These results show that manure has a negative effect on 17.461 but a positive effect on FOS8500. The negative effect on yield due to application of manure is increased when the corn is grown on poorer soils in an arable system.

5) Another set of field trials compared FOS8500 with various hybrids of varying maturities made with the putative N2 fixing inbred C4-6 and another set made with the Mandaamin inbred NG10. The corn yielded 28 bu/acre more in the cattle system. Though results were not significantly different, the interaction between manuring and hybrid type showed negative responses of the C4-6 hybrids to manuring but positive responses of the NG10 hybrids and FOS8500 to manuring.
6) A major question arising from this research is why the C4-6 hybrids respond negatively to manure on the poorer soils in the arable cropping systems? A study on such a soil on the Beiler farm showed that manuring decreased grain yields 20%, nitrogen uptake 32%, uptake of macronutrients 20-22%, and especially total uptake of micronutrients 46% across the C4-6 based hybrids. The commercial hybrid and other kinds of Mandaamin hybrids responded positively to manuring with 38% more yield, 3% more N uptake, 8-16% more uptake of macronutrients, but 19% less micronutrient uptake. Strip trials with and without manure were also carried out on the Weiss/Bauer farm, which had a very high soil fertility due to repeated applications of dairy manure. The 17.461 did respond negatively to manuring with a 19% reduction in grain yield and a 6% reduction in N uptake. However the effect of manure was positive for uptake of other nutrients. The differences between the response on the Beiler and Weiss/Bauer sites could relate to the necessity for hybrids like 17.461 to exercise rhizophagy on soils with lower nutrient availability, and that they are prevented from doing that when manure is applied.
7) Other field studies indicated that grain yields were 10-11% lower for the highest yielding Mandaamin hybrids with comparable maturity relative to conventional hybrids when grown under manured conditions. This confirms results from a multiyear OREI study in Illinois, Indiana, and Wisconsin which is otherwise not described here but which overlaps this study. The Mandaamin hybrids of comparable maturity to commercial checks had generally lower harvest index values but they had higher stover (stalks + leaves + husk) and root production.
8) Analysis of data from OREI and SARE trials showed a higher methionine, lysine, carotenoid, and mineral content in the grain of the Mandaamin hybrids. This also confirms results from the multiyear OREI study. The commercial check had more starch but less oil and protein in the grain and their protein was of poorer nutritional value.
9) Diet modelling with a cooperating poultry scientist (not shown in this report) that considered methionine contents showed the same value per acre for the organic poultry feeder of the

Mandaamin crop as for the commercial organic hybrid, despite 11% less yield. This was because the higher content of methionine in the Mandaamin hybrids allowed for replacement of expensive organic soybean meal in the ration.

10) On farm studies with the Mandaamin hybrids showed that they had increased acquisition of macronutrients and microelements from the soil relative to conventional hybrids and higher contents of minerals and other nutrients in the grain. The magnitude of this enhancement in mineral uptake and concentration in plant tissues varied with sites, hybrids and years. In 2019 the relative values were calculated by taking the ratio of least square mean values between Mandaamin hybrids and the commercial check. The concentration of nutrients in grain for three Mandaamin hybrids ranged from 6 to 14% higher for macronutrients and 6-30% higher for micronutrients than the check. Total uptake ranged between Mandaamin hybrids from 5-19% more for macronutrients and 14-20% more for micronutrients. Ratios calculated using raw data showed the Mandaamin hybrids ranging from 12-24% higher than the check across all nutrients. The highest value in almost every case was for the 17.461 hybrid. In 2020 ratios based on raw data showed that nutrient concentration in grain across 8 different Mandaamin hybrids averaged 20% higher than the check. The 17.461 again had the highest mineral contents.
11) Natural isotope abundance studies of the Mandaamin hybrids indicated enhanced N_2 fixation. This paralleled increased mobilization of nutrients from soil into grain. Both processes seemed to be linked.
12) The Mandaamin corn hybrids differed from each other in their abilities to acquire N from microbial biomass/organic matter or to fix N_2. In 2019 trials the hybrids that had C4-6 in their parentage appeared to extract the most N from air and microbial biomass/soil organic matter. They also took up the most minerals and partitioned more into grain. The 2020 trials showed the critical importance of the other half of a C4-6 hybrid in stimulating nutrient uptake, partitioning of nutrients into grain, and yield.
13) The natural abundance method for assessing nitrogen fixation utilizes differences in relevant $\delta^{15}N$ values that indicate the relative amounts of natural ^{15}N and ^{14}N isotopes in the total N. In 2019 results supported the hypothesis that the active rhizophagy cycles in the Mandaamin hybrids cause higher $\delta^{15}N$ (i.e. more ^{15}N) in root and stover. This occurred again in 2020 but also in grain. Based on the scientific literature it is most probable that the enhanced ^{15}N uptake is sourced from microbial biomass and easily extractable organic N made up of microbial necromass. In 2019 the ^{15}N-rich N taken up from the soil appeared to be progressively diluted from stalks to grain, leading to greater ^{14}N enrichment in the grain and strong decreases in $\delta^{15}N$. This may have been associated with absorption of depleted N occurring as a result of N_2 fixation in the Mandaamin hybrids, that possibly took place in foliar tissues (Micci et al., 2022). Hence, in the best N efficient hybrids the $\delta^{15}N$ levels may be simultaneously increased and decreased in the plant parts by these competing plant/microbial activities.
14) The percent N or protein in grain and $\delta^{15}N$ in grain proved to be the two central and inter-related indicators of crop performance. Both $\delta^{15}N$ and %N correlated strongly with each other and positively with uptake and concentration levels for numerous minerals as well as for grain quality parameters including essential amino acids, starch, oil, and density. In multi-site trials in

2019 and 2020 analyses showed the majority of variation in $\delta^{15}N$ was associated with %N (or % protein) in grain, and the converse was also true.

15) The balance of fixation and acquisition from soil microbial biomass/organic matter depended on hybrid, farm history, soil quality, and year of trials. The highest estimates for fixation level of N_2 into grain (48%) and acquisition of N from microbial biomass/easily available organic matter (58%) was estimated for the C2B2.C46 hybrid when grown under organic arable systems in 2019. However, the other Mandaamin hybrids tested appeared to fix or extract more N from biomass or the air when they were grown under the organic or conventional cattle systems.
16) In 2019, rhizophagy seemed to predominate for the Mandaamin hybrids as a source of N for grain in the arable organic system specifically on the organic-arable system's N depleted soils. Similarly, in 2020, the $\delta^{15}N$ results in 2020 showed that rhizophagy predominated in its effects on grain protein-N loading, possibly obscuring inputs from fixation. The growing conditions in 2020 were more stressful and resulted in lower yields and harvest index values for the varietal trials. In this case stover generally had a lower $\delta^{15}N$ value than grain. A possible explanation for these differences from 2020 may be less fixation of N from the air in the foliage in 2020 than in 2019 due to stress conditions. Also, the C2B2.C46 hybrid, which had appeared to fix large amounts of N_2 even on the poorer, arable organic soils in 2019, was not included in the 2020 trials.
17) In the 2020 variety trials the uptake of minerals, C, and N into grain in 2020 was positively associated with $\delta^{15}N$ level of the grain but negatively associated with the $\delta^{15}N$ level of stover. Similarly, the mineral uptake in the stover was positively associated with the $\delta^{15}N$ levels in the stover, but negatively associated with the $\delta^{15}N$ levels in the grain. This reciprocal relationship can be explained as a source-sink relationship. The grain is a sink for N^{15} enriched N and minerals that are loaded into the grain from the stover source which depletes the stover.
18) In both years the C4-6 based hybrids took up the most minerals, and also had high $\delta^{15}N$ levels in their stover and higher %N in their grain. In 2019 when roots were analyzed, the C4-6 hybrids had exceptionally high microelement contents in their roots. In both 2019 and 2020 trials, 17.461 produced grain with high mineral contents. In both years it produced large quantities of stover and demonstrated the strongest ability to acquire minerals and to partition micronutrients into grain. In the 2020 varietal trials 17.461 averaged 61% higher mineral contents in its grain than the check. This difference was exceptionally high. It included 270% more Ca, 99% more Cu, 10% more Mg, 41% more Mn, 9% more P, 19% more S, and 36% more Zn. As yields of 17.461 were very similar to FOS8500 these very large differences are clearly not due to yield reduction coupled with some kind of nutrient concentration effect.
19) To evaluate the partitioning of minerals into grain we calculated a harvest index value for each mineral (harvest index = (mineral in grain/mineral in grain + stover)x 100).In the 2020 varietal trials the $\delta^{15}N$ in grain and stover was associated with a major part of the variation in the harvest index of individual minerals. As had been the case for mineral uptake into grain, harvest indices for minerals were positively associated with the the $\delta^{15}N$ levels in the grain but negatively associated with the $\delta^{15}N$ levels in the stover. This again makes sense as the outcome of a sink-source relationship with the assumption that the movement of N derived from rhizophagy into grain is coupled with enhanced mineral uptake derived from rhizophagy into

grain. Hence the harvest index and mineral uptake are probably linked to the strength of exercise of the microbial partnership.

20) Studies with different kinds of soil tests in 2019 showed that the Mandaamin hybrids had the highest yields and nutrient uptake when they were grown on cattle manured soils with high levels of total organic-N, high C/N ratios and high soil protein content. Hence management oriented towards building organic-N and soil protein may be determinative for enhancing stabile, high level agronomic performance of the Mandaamin hybrids.
21) Uptake of specific mineral nutrients varied from year to year and site to site. The high uptake of certain minerals by the Mandaamin hybrids in 2019 studies suggests enhanced mobilization of minerals from the solid portions of the soil including alumino-silicate clay particles, from parent mineral particles, and from limestone rather than from passive reliance on the soil solution.
22) Multiple experiments showed that inoculation with N_2 fixing bacteria did not improve performance of Mandaamin hybrids but inoculation may have had some benefits on the performance of conventional hybrids.
23) The inoculation and manure trial results suggest that the Mandaamin hybrids have established internal microbial cultures and that addition of bacteria to the seed or soil in the form of manure may interfere with rhizophagy and hence, crop performance.
24) The negative response to direct manuring and the lower nitrate and available amino acids found before harvest under the Mandaamin hybrid roots suggest lower nitrification under their roots and lower reliance on mineral N nitrification from soil and manure.
25) Future studies might be carried out to test whether the suppositions derived from this research remain valid with more data gained after larger-scale application of the Mandaamin hybrids on farms. We are at the beginning of learning about relationships between breeding, rhizophagy, seed-borne endophytes, soil fertility, soil microbial biomass and microbial necromass, N fertilizers and yield. It is important to gather more data comparing N need, utilization, and fertilization of soil under different farming systems and learning how to optimize hybrid partnerships with endophytes. And finally, it is important to continue breeding for hybrid/endophyte partnerships with greater stability for both microbial assisted processes of rhizophagy and N_2 fixation coupled with adequate yield performance and enhanced nutritional value under conditions with reduced N inputs.

Background.

Evolving working hypotheses: This project became part of a dynamic, quickly evolving, and novel research paradigm. Soon after the inception of the research we became involved in testing our inbreds for seed borne endophytes together with James White at Rutgers University. Dr. White has pioneered research on such endophytes which in most wild plants enhance mineral uptake, growth, and health through the rhizophagy cycle (White et al 2018; 2019a, 2019b). In that cycle seed borne microbes are multiplied in roots, the bacterial cell walls and a portion of total bacterial bodies are consumed, and the bacteria are subsequently excreted through root hairs into the rhizosphere. The bacteria replenish their cell walls in the soil but may be taken up again by growing roots where they are again partially consumed and excreted. The likely biochemical dialogue that underpins the cycle is described in Chang et al. 2021.

Dr. White responded to the publication of our earlier research (Goldstein et al, 2019) by contacting us. We began cooperative research and discovered that that Mandaamin varieties (but not conventional varieties) were carrying gangs of seed borne bacterial endophytes in their seed. These bacteria were excreted from root hairs into the surrounding rhizosphere in relatively large amounts through the root hairs of our plants (see photo collage 1). Photos presented here are from seedlings that were grown axenically in Petri dishes in sterile agar following a light surface disinfection of seed. All inbreds were grown in the same nursery in 2019. Photo collage 2 shows the formation of bacterial aggregates in putative N2 fixing inbred C4-6 and bacteria and spore formation (transparent spheres) in the rhizophagic inbred C2B2. Photo collage 3 shows root tissues of conventional inbreds LH206 and Novartis 942 which showed no signs of bacterial colonization of roots. However, roots of seedlings from Novartis 942 showed colonization with fungal hyphae.

Photo collage 1.

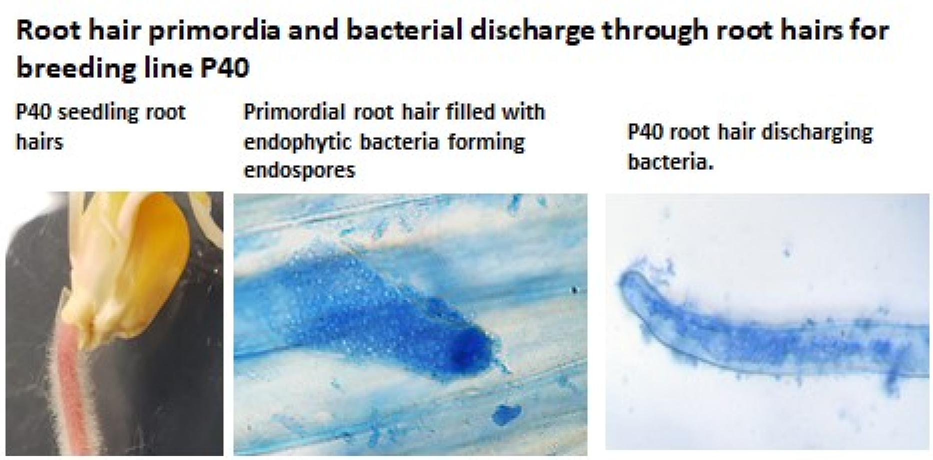


Photo collage 2. Bacterial aggregates in the roots of the C4-6 inbred (left) and spore forming bacteria in C2B2.

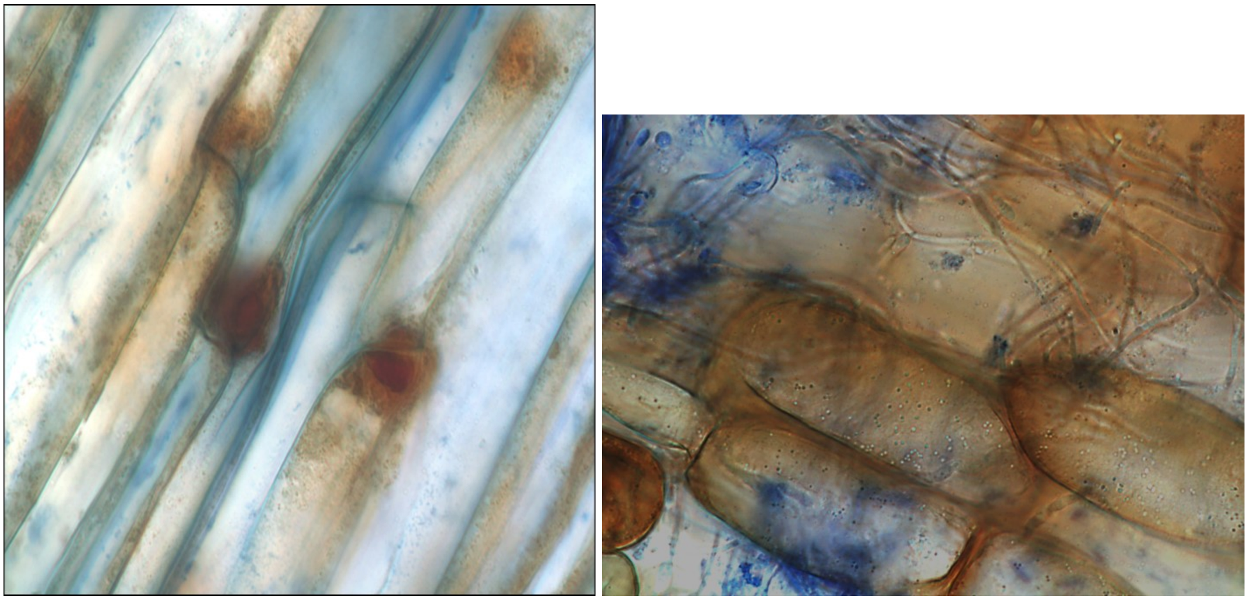
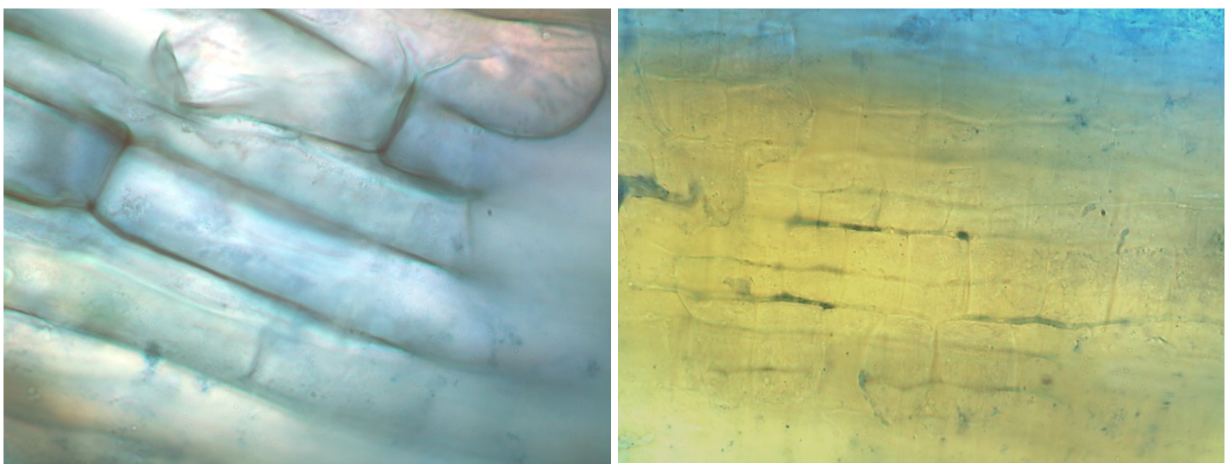


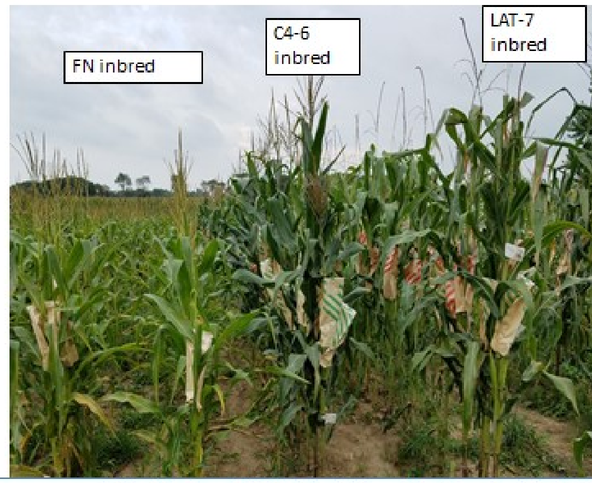
Photo collage 3. Roots of LH206 on left and of Novartis 942 on right.



This classical demonstration of rhizophagy has been shown in other plants to increase mineral uptake and to induce root branching, root hair production, and incorporation of nitrogen from the air (White et al., 2018; 2019a, 2019b). More recent work by the White lab, with numerous plant species, has revealed the nature of the biochemical dialogue between microbes fostered in growing shoots and reproductive organs (Chang et al., 2019; Micci et al., 2022). These endophytic bacteria are fostered and fed by plants in the area around plant hairs (trichomes). Biochemical studies with various stains have confirmed parts of the hypothesis that these microbes, working in the shoots and reproductive organs of different non-leguminous species, are engaged in N_2 fixation. The quantity of N_2 that can be fixed by such endophytes is presently unknown, but initial studies suggest more N is taken from the air in shoots than in roots (Micci et al., 2022).

The results from the White lab have informed our thinking because they could help explain many of the phenomena discovered in the course of this project described below relating to mineral uptake and N acquisition. For example, the putative N_2 fixing inbred C4-6 developed at the Mandaamin Institute (Photo collage 4) and used in the trials below, and other inbreds as well, have relatively hairy leaves and stalks as well as highly branched, relatively disease free rooting systems (photo collage 5 and 6).

Photo collage 4 shows hairy stalks of C4-6 on left and foliage color of C4-6 when grown beside a normal inbred on left (FN) and LAT-7, an inbred derived from root weeping landrace Mixeno.



Phenotype of N efficient/N2 fixing inbreds. Results in 2019 with corn inbreds following after a winter rye disk down crop. FN is a typical inbred. The pedigree of LAT-7 is 1/4th Mixeno.

Photo collage 5 shows rooting systems of multiple commercial or Mandaamin inbreds grown on N limited sites in 2017 without fertilization.

Conventional inbreds LH206, LH123, S7, S5 grown on JR or Creek field (S5) in 2017 without fertilizer. LH206 and 123 were bred by Monsanto; S7 and S5 are commercial inbreds from a seed licensing company.

Nokomis Gold Seed Company inbreds C4-6, LAT-7, NG2-3-2, and C2-B bred at the Mandaamin Institute and grown on JR or Creek field (C2-B) in 2017 without fertilizer.

Photo collage 6. Roots extracted in September from Mandaamin nursery show root health of putative N2 fixing inbred C4-6 (left) and conventional inbred LH206 (right). The two hybrids possess the same relative maturity.

Our ideas about nitrogen fixation have also evolved in the course of this project in consonance with the results of our studies. Unequivocal confirmation of nitrogen fixation in the field is difficult to achieve. The $\delta^{15}N$ value assesses the ratio between the two natural isotopes ^{14}N and ^{15}N. The higher the value, the greater the concentration of ^{15}N. Soil microbial biomass and extractable organic N mostly have higher ^{15}N content than bulk soil and air and hence high $\delta^{15}N$ values (Craine et al., 2015). This is because bacteria consume soil organic matter and through isotopic partitioning they release low $\delta^{15}N$ nitrate and ammonium into the soil while retaining more ^{15}N in their bodies. They also selectively release more ^{14}N than ^{15}N into the atmosphere during denitrification.

As rhizophagy involves the oxidative destruction of microbes to obtain nutrients it can be assumed that N uptake in plants that have active rhizophagy will be skewed in favor of ^{15}N. Our previous studies (Goldstein, et al., 2019) showed that the grain resulting from many of our inbreds and populations had higher $\delta^{15}N$ values than conventional inbreds and hybrids, suggesting that they obtainin a portion of their N from microbial biomass and easily extractable organic N. But the grain of very high chlorophyll, putative N fixing populations had much lower $\delta^{15}N$ values indicating that they were getting up to half of their N from the air.

An alternate explanation to explain difference in plant $\delta^{15}N$ values might be fractionation of nitrogen isotopes in the plant. In some cases fractionation might occur due to the enzymes nitrate reductase, nitrite reductase, and glutamate and glutamine synthase (Craine et al., 2015). But this kind of fractionation, if it does occur in vivo, would discriminate against ^{15}N and would therefore be expected to reduce the ^{15}N content of grain, not increase it. Furthermore, according to Craine et al (2015) ***"If the major N source for plants in soils is inorganic N, the $\delta^{15}N$ of plants should more closely correlate with the $\delta^{15}N$ of that source than total N. Although a more comprehensive survey and broader sampling are required, published values of foliar $\delta^{15}N$ largely reflect the signatures of inorganic N available in soil. The vicinity of most plots to the identity line indicates that, in most non-boreal sites, plants mainly acquire NH_4 + and NO_3 − from soil and this uptake occurs without any large isotopic fractionation."*** Figure 3 in Craine et al (2015) shows a compilation of data from multiple authors that indicates a tight relationship between the ^{15}N composition of inorganic N and the ^{15}N content of foliage. Hence it seems most likely if the Mandaamin corn has a higher ^{15}N content in its body than neighboring check varieties then it probably relies at least partially on a source of N nutrition that has a higher ^{15}N content. In soils, this ^{15}N enriched source is probably microbial biomass or easily extractable organic matter composed of decomposing microbes.

Based on the evidence and inferences above it is assumed that 1) the Mandaamin varieties have active rhizophagy cycles. 2) Rhizophagy causes higher $\delta^{15}N$ by increasing N uptake from microbial biomass and easily extractable organic N. 3) As roots are probably the most active site for microbial turnover, a high $\delta^{15}N$ should be especially apparent in roots. 4) In the cases where N fixation is also fostered by foliar endophytes, $\delta^{15}N$ is lower in the sink organs such as grain due to a ready supply of depleted $\delta^{15}N$ in the form of nitrate or ammonium. The ^{15}N enriched N taken up from through rhizophagy would be progressively diluted with ^{15}N depleted N from atmospheric fixation, leading to lowered $\delta^{15}N$ values due to greater ^{14}N enrichment from fixation. This lowered $\delta^{15}N$ might be especially apparent in grain.

Hence in the best N efficient hybrids the $\delta^{15}N$ levels might be simultaneously increased and decreased in the plant parts by these competing plant/microbial activities. It seems probable that the overall balance may shift from growing phase to growing phase and from year to year depending on growing conditions and plant allocation of resources to partnerships, and that one process may obscure the other from the standpoint of $\delta^{15}N$ assessments. Furthermore, it is expected that $\delta^{15}N$ values would shift depending on whether the microbes are predominantly engaged in N_2 fixation and supplying nitrate or ammonium to the plant with depleted $\delta^{15}N$ or they are being consumed by the plant providing materials enriched in $\delta^{15}N$.

Methods: The experiments carried out between 2018 and 2021 during this grant comprised mainly two types of trials. The first were the SARE funded trials to examine the effect of manure and inoculation with nitrogen fixing bacteria on the yield and quality of single N efficient hybrids relative to conventional hybrids. The second major emphasis was on examining the performance of multiple hybrids on different organic and conventional farm sites, with and without manuring. The latter effort was jointly funded with OREI and SARE.

The sites chosen were on twelve working farms located from Southeastern to Northwestern Wisconsin. Dr. Micheal Travis (UW Extension-Pepin County) and Chase Cummings (Pepin County Conservationist) took part in guiding and conducting the research in the Durand/Arkansaw area. Farmers performed the research by tilling, planting, weed control. Wisconsin farmers Anibas (Arkansaw), Beiler (Rewey), Clark (Spring Green), Doudlah (Evansville), Esch (Fennimore), Adsit (East Troy), Egre (Cambridge), Michael Fields Agricultural Institute (Troy), Anon (Darlington), Weiss/Bauer (Durand), and Zinniker (Elkhorn) participated in the research, in a gradient stretching from the Southeast to the Northwest portions of the state. Research was also carried out on the Lengacher farm in NE Ohio and on the Michael Fields Agricultural Institute farm in Elkhorn, WI. Farm Weiss/Bauer was conventionally managed monoculture corn with reduced chemical inputs but with a history of large inputs of dairy manure. The other sites were organically managed. The rotations and the inputs for 2019 are shown for several of the farms in Table 1.

Inclusion of these farms allowed us to examine and contrast the performance of the Mandaamin hybrids under farm conditions with different kinds of management histories. The Adsit, Egre, Beiler, and Doudlah farms are organic operations that are largely based on arable cropping. The Adsit and Doudlah farms rely on bought in chicken or other livestock manure. The Anibas, Zinniker, and Anon farms are longer term organic cattle based farms with some arable cropping, perennial forage, and ample cattle manure applications.

Research was carried out in strip plots planted on the farms, with various amount of replication. Strip plots ranged from 40 feet to several hundred feet in length and were two rows wide on the Beiler, Zinniker, and Anon sites, respectively, and three rows wide for Anibas and Clark. Spacing between rows was 2.5 feet for farms Adsit, Beiler, Clark, Egre, Stoltzfus, Michael Fields Agricultural Institute, and Weiss/Bauer, 3.167 feet at Zinniker, and 0.125 feet at Doudlah.

Varietal trials consisted in plantings of a set of Mandaamin hybrids and commercial hybrid checks. Row numbers per plot generally varied generally from two to four rows. Distance between rows was generally 30 inches and planting density was generally 30,000 plants/acre. Various hybrids were compared on these farms. Though the hybrid identity varied from year to year, 17.461 (105 day) and 17.2B24 (108 day) were used as constant Mandaamin checks each year alongside commercial hybrid checks. The commercial checks consisted of Foundation Organic hybrid FOS8507 (110 day maturity) in

2018 or FOS8500 (105 day maturity) in 2019, 2020, and 2021. In some of the studies, varieties were planted across manured and non-manured strips. With few exceptions, strip plots were generally unreplicated on each site but a randomized version of the same design was planted on all farms within a given year.

2019 varietal trials: In 2019 hybrid comparisons were made on 7 farms, but study also focused effort on five farms to study N cycles on a subset of four hybrids across three different farming systems. The four hybrids chosen were the FOS8500 control, 17.461, 17.2B24, and C2B2-1.C46. The latter three Mandaamin experimental hybrids were essentially derived by crossing three parents: 17, C4-6-1, and C2B2 (17.461=17 x C4-6-1; 172B24=17 x C2B2;. C2B2-1.46=C2B2-1 x C4-6-1).

Table 1 describes sites used for varietal trials in 2019.

Farm	Year	Crop rotation (past 4-5 years)	Soil fertility in corn phase	Soil fertility time	Soil texture	manure rate
Clark	**2018**	**alfalfa-corn**	**+/- cow manure**			**10 tons/acre**
Stoltzfus	2018	Wheat-Hay (alfalfa+clover+timothy)-Corn	CC	Preplant	Silty clay loam	0
Adsit	2019	Alfalfa (3 years)-Wheat-Corn	Chicken manure	Preplant	Clay loam	3 tons/acre
Doudlah	2019	Soybean-Corn-Kidney bean	CC	Preplant	Silty clay loam	0
Zinniker1	2019	Hay (grass+alfalfa for 5 years)-Corn	Compost	Preplant	Silty clay loam	15 tons/acre
Anibas	2019	Alfalfa (3 years)-Corn	Cattle manure	Preplant	Silty clay loam	6000 gal/acre
Beiler1	2019	Oat-Corn-Pumpkin-Corn	Chicken manure	Preplant	Silty clay	10 tons/acre
Stoltzfus1	2019	Hay-Hay (alfalfa+clover+timothy)-Corn	Cow manure slurry + box stall manure	Preplant	Silty clay	4800 gal/acre + 660 bu/acre
Stoltzfus2	2019	Hay-Hay (alfalfa+clover+timothy)-Corn	Cow manure slurry	Preplant	Silty clay	7000 gal/acre
Weiss/Bauer	2019	inoculation trial continuous corn	N starter +/- slurry	Preplant	sandy loam	75 lbs N starter, +/-unspecified slurry
C.C. means cover crop.						

In preceding years the C2B2-1.C46 routinely appeared to show the greatest signs of being N efficient by its robust early growth coupled with very dark foliage and high chlorophyll scores, and Mandaamin Institute breeders assumed it to be the most likely to be fixing N_2 (see photo 2 below). 17.461 appeared quite similar, but not as extreme in its coloration and early vigor. Of the three, 17.2B24 seemed to have more normal early growth and chlorophyll scores and it was rated as being efficient at obtaining N from organic matter but not necessarily N_2 fixing.

Photo 1. C2B2.C46 growing on the Zinniker Farm in 2017.

2020 varietal trials: In 2020, nine different hybrids were grown in similar strip trials on multiple sites. The assortment consisted of the FOS8500 check 17.2B24, 17.461, and six mostly early Mandaamin hybrids, four of which had received only minimal testing in the past. The hybrids ranged from 92 to 108 day relative maturity. The trials took place on two adjacent, manured and non-manured sites on the Beiler farm, on a non-manured site on the Clark and Egre farms, on a manured site on the Esch and Weiss/Bauer farms, and on a not manured site on the Lengacher Farm. The Egre farm site was largely decimated by deer. The Clark farm had too much variation in soil moisture gradients from subsoil seepage to give reliable results. The Lengacher farm produced valid grain yields, but because the farm was located in Ohio we were unable to get full data on plant populations and stover yields. We gained full sets of data from the two sites at Beiler, and single sites at Esch, Goldstein, and Weiss/Bauer farms where corn followed after pumpkins strawberries, alfalfa, and corn, respectively. Grain and stalk samples were sent to USDA in Morris Minnesota for determination of mineral contents in grain and to UC Davis for isotope analyses. Grain samples were sent to the Grain Testing Lab at Iowa State University for determination of grain nutritional value, density, and ethanol yield.

Table 2 describes sites used for varietal trials in 2020 and provides notes on weeds and animal damage.

Farmer	Plot dimensions			Preceding crop				Tillage				biology			notes
	no rows	row width inches	row length (feet)	2019	2018	2017	2016	planting date	primary tillage	secondary tillage	weed control	weeds	weed control	manure	
Moses Beiler	2	30	100	alfalfa&grass	alfalfa&grass	alfalfa&grass	corn	ca. May 15	spring plow	disk 3x	drag 1x; cultivate 2x	foxtail, quackgrass	fairly good	with and without ca 10t/a BYM	Good stands and yields.
Daniel Esch	2	34	60	strawberries	strawberries	strawberries	pasture	end May	spring plow	harrow cultimulch 3x	cultivate 3x	fairly clean	good	12-15 t/acre composted dairy pack	Good stands and yields.
James Lengacher	2	30	30	legume grass hayfield	legume grass hayfield	legume grass hayfield	soybeans	June 8th	deep rip 8 inch tine	disk 2x; field cultivator	cultivate 2x	practically no weeds	excellent	2019 hay got 4-5 t/a manure/compost	Population density not determined.
Dale Clark	3	30	42	fallow & weeds	fallow & weeds	fallow & weeds	fallow & weeds	22-May	offset heavy disk	not necessary	rotary hoe 1x cultivate 1x	mostly foxtail	good	unmanured	spatial variation, erratic stands, poor growth in very low fertility spots; variable moisture; erratic yields, not usable.
Jim Egre	2	36	50	soybeans	grass	grass	grass	June 5th	plowed	rotary tiller 3x	cultivator + hand weed	foxtail, creeping charlie, morning glory	good	unmanured	Plano silt loam B soil. Deer severely damaged plots so a yield determination was not feasible.
Gold/Mandaamin/MFAI	2	30	40	alfalfa&grass	alfalfa&grass	alfalfa&grass	alfalfa&grass	June 16th	offset disk 3x		rotary hoe 1x cultivate 2x	Canada thistle patch	poor	not manured	Severe Canada thistle infestation held in check by cultivation.
Gary Bauer/Don Weiss	3	30	90	corn	corn	corn	corn	6-May	vertical till		herbicide	some velvetleaf	good	not manured	

Manure/inoculation trials. The experimental design generally used in manure/inoculation trials from 2018 to 2020 was a split block design with manuring being the first split applied to half of the experimental area. Manure application was targeted at 10 t/acre of livestock manure. Superimposed across this in the form of strip plots was a 2x2 factorial design consisting of two hybrids which were either inoculated or not inoculated with a proprietary mixture of nitrogen fixing bacteria which originated from the Mandaamin Institute in conjunction with the inoculate company TerraMax. These treatments were laid out as strip plots across manured and non-manured treatments, and they were replicated three times. In several cases the experiment was modified by not including the manure application. In other cases the entire field was manured.

In the SARE funded inoculation trials, two hybrids were always compared, a Mandaamin hybrid and a check. In 2018 the two hybrids were a commercial hybrid from Foundation Organic Seed (FOS8507) with a relative maturity of 110 days and an experimental Mandaamin Institute hybrid (C461.C2B2) of the same maturity with putative N efficiency. During 2019 the experimental Mandaamin hybrid 17.461 was compared with the commercial FOS hybrid FOS8500. These hybrids both possess a relative maturity rating of 105 days. During 2020 the experimental Mandaamin hybrid 15.461 was compared with the commercial FOS hybrid FOS8500. These hybrids both possess a relative maturity rating of 105 days. The FOS8500 and 8507 represented the highest yielding hybrids available from the Foundation Organic Seed company. They were based on seed available from reputable commercial seed brokerage companies and have tested at the top of yield trials on multiple sites (personal communication, 2018, Steve Mohr, owner Foundation Direct Seed company.

Soil samples Our intent was to capture any different effects soil quality might have on the different hybrids and their N efficiency/N2 fixation, and the effects the hybrids might be having on soils. Towards that ends, samples were taken before planting in some of the varietal trials and manure/inoculation trials. After planting soil samples from the organic farms were gathered from the varietal trials and analyzed at the University of Illinois by as part of the OREI funded project there. Furthermore, with SARE funding in 2019 we sampled OREI and SARE sites in September before harvest under selected hybrids to examine soil quality and relationship to crop performance. Soil samples for these trials were taken to a depth of 8 inches under four hybrids in the OREI trials and under two hybrids in the SARE trials. Samples were sent to Woods End Soil Testing Lab in Maine for soil quality measurement and to Cornell University for determination of soil protein content.

Plant samples. To determine population density and grain yield, ears and population data were gathered on three 8.75 foot samples that were taken from each plot at regularly spaced sampling sites of approximately 30 feet apart. On the Doudlah farm, two samples of that length were taken on each of three sites sampled. Three stalks from each of the sample sites were pooled across plots, weighed, ground and subsequently tested for moisture content in order to determine the dry matter yield of stalks. A 12 x 12 x 8 inch deep soil/root monolith was extracted from a centered crown of one plant on each sample site. Root samples were soaked, spray washed and dried to determine dry matter content in roots. Grain yield was calculated assuming 56 pounds of grain per bushel, and results were standardized for grain moisture of 15.5%. Grain, stalk and root samples were shred, mixed and subsamples were ground with a Wiley mill. Samples were sent to the USDA-ARS North Central Soil Conservation Research Lab in Morris Minnesota (Abdullah Jaradat, Jane Johnson, Chris Wente) for analysis of mineral content using ICP and for N and C content using a Leco elemental analyzer. Nutrient values were analyzed both in terms of tissue concentration and total plant uptake. Samples were further ground, mixed, and packed by USDA into capsules and sent to the UC Davis isotope facility for analysis of ^{14}N and ^{15}N isotopes and for determination of the $\delta^{15}N$ ratio.

Initial studies with isotopes in 2009 and 2010 showed that the grain resulting from inbreds and populations from the breeding program had higher $\delta^{15}N$ values than conventional inbreds, but that the grain of N fixing populations had much lower $\delta^{15}N$ values. We assume that rhizophagy causes higher $\delta^{15}N$ by increasing N uptake from microbial biomass and from easily decomposable organic matter (Craine et al. 2015). We also assume that in some cases, where N fixation is fostered by endophytes, the grain $\delta^{15}N$ is lower than for conventional inbreds because the N taken up from the soil is progressively diluted with N from the air, leading to greater ^{14}N enrichment. Hence in the best N efficient hybrids the $\delta^{15}N$ levels are being increased and decreased in the plant parts by these competing plant/microbial activities.

Based on these suppositions, nitrogen derived from air (NDFA) for the grain of the different hybrids was determined in two different ways; internally, contrasting $\delta^{15}N$ (delta 15N) values for the parts of a given hybrid between the high values found in roots or stalks and the low values found in grain; or by subtracting the $\delta^{15}N$ value for a given Mandaamin hybrid from the check, dividing the product by the $\delta^{15}N$ value for the check, and multiplying that result times 100. Application of these formulas for determining nitrogen acquired from soil/microbial biomass/organic matter or from the air by the Mandaamin hybrids relative to the FOS hybrid are explained in the results section.

Data analysis Data was analyzed with Excel spread-sheets and JMP software. Response factor data was explored utilizing linear models, analysis of variance, means generation, and means separation. Where appropriate an analysis of variance included relevant covariates for partitioning out contributing variation. In several cases, population density was utilized when necessary as a covariate to compensate for differences in plant stands, but other correlative factors were used where appropriate to clarify sources of variation for $\delta^{15}N$ scores. In such cases adjusted least square means were generated and various models were compared for their effects on explaining total variation and estimates of fixation. Analyses of variance were often partitioned for the % of the total SS (sums of squares) attributed to a given factor. Analyses were made of roots, stalks plus leaves (stover), and grain. Harvest index values were calculated as yield in grain/total yield of grain and stover) x 100. Indexes were calculated for grain yield in 2019 and for grain yield and uptake of minerals in 2020 varietal trials.

The strip plot design used for the variety trials was un-replicated within sites but the farms served as replicates. This basic design does not allow testing for farm differences in the farm x hybrid interaction as that latter interaction is used as an error term to test both farms and hybrids. However, the types of farms we were testing fell into three very different groups. These were organic cattle-based farms which utilized their cattle manure and forages in their rotations alongside production of corn and small grains (Anibas, Anon, Zinniker). The organic arable group consisted in farms that mainly utilized chicken manure to sustain mostly arable production (Adsit, Beiler, Doudlah), though forage crops were also grown occasionally at Beiler's. The third group consisted of two conventionally managed fields (Weiss and Bauer) with monoculture corn that utilized inputs of starter fertilizer and herbicides and had a history of high inputs with cattle slurry from a dairy operation. In 2019 we tested data from two sites in each of these three types (arable organic=Beiler, Doudlah), (organic cattle = Anon and Zinniker), and conventional manured corn = two fields at Weiss/Bauer). On the Doudlah site chicken manure was not applied in 2019. On the Weiss/Bauer fields, one received only starter fertilizer; the other received starter and manure. Analyzing data by type of farm enabled us to test for significant effects due to type of farm, hybrid, and type x hybrid. Due to the levels of variation inherent in the on-farm research we did, we considered p values to be worthy of discussion if they were approximately 10% or less.

Results:

Over all yields.

Throughout the experiments the hybrid 17.461 characterized itself as probably the best adapted of the Mandaamin hybrids that possessed the same relative maturity (105 days) as the commercial hybrid check FOS8500. Table 3 summarizes overall yield data from 14 strip trials on different farms where 17.461 was compared with FOS8500. More information on these two hybrids is presented in sections below from specific trials. Here we are comparing yields across two years and many different kinds of farms, farming systems, and climatic factors. There were 4 sites where comparisons were made in the context of an arable organic cropping system that was manured, and 3 sites where it was not manured. There were five sites where comparisons were made in the context of a cattle farming operation with manure applied but two sites where manure was not applied. Due to this structure, and the need to make relevant comparisons in the context of different conditions on the different farm sites, the 17.461 yields were converted to a percentage of the FOS8500 yield on the same site. These percentages were then analyzed using an analysis of variance with system, manuring, hybrid and all the interactions of these factors. Doing this standardized the results so the focus was on the relative difference between the two hybrids irrespective of the general yield level.

Table 3. The yields of corn on 14 sites comparing yields of FOS8500 and 17.461.

year	farm	system	manuring	hybrid	Grain yield bu/acre	%
2019	Doudlah	arable	none	FOS8500	59	100
2019	Bauer/Weiss	cattle	none	FOS8500	177	100
2020	Bauer/Weiss	cattle	none	FOS8500	145	100
2020	Lengacher	arable	none	FOS8500	113	100
2020	Beiler	arable	none	FOS8500	94	100
2019	Doudlah	arable	none	17.461	98	166
2019	Bauer/Weiss	cattle	none	17.461	180	102
2020	Bauer/Weiss	cattle	none	17.461	131	90
2020	Lengacher	arable	none	17.461	124	110
2020	Beiler	arable	none	17.461	128	136
2019	Zinniker	cattle	manured	FOS8500	200	100
2019	Anibas	cattle	manured	FOS8500	150	100
2019	Anon1	cattle	manured	FOS8500	253	100
2019	Anon2	cattle	manured	FOS8500	228	100
2019	Beiler	arable	manured	FOS8500	173	100
2019	Adsit	arable	manured	FOS8500	81	100
2020	Beiler	arable	manured	FOS8500	117	100
2020	Esch	arable	manured	FOS8500	228	100
2020	MFAI	cattle	manured	FOS8500	50	100
2019	Zinniker	cattle	manured	17.461	207	104
2019	Anibas	cattle	manured	17.461	132	88
2019	Anon1	cattle	manured	17.461	227	90
2019	Anon2	cattle	manured	17.461	164	72
2019	Beiler	arable	manured	17.461	122	71
2019	Adsit	arable	manured	17.461	79	98
2020	Beiler	arable	manured	17.461	94	80
2020	Esch	arable	manured	17.461	207	91
2020	MFAI	cattle	manured	17.461	53	106

The analysis of variance of the data converted into relative values shows no significant factors for analysis of the raw data. However, when the data was adjusted as % of the FOS8500 control there were significant effects for all factors except hybrid . In the latter analysis there were identical sum of squares for manuring and manuring x hybrid as well as for system x manuring and manuring x hybrid x system. Highlights of the yield trials were that:

- The 17.461 averaged 139 bu/acre and the FOS averaged 144 bu/acre.
- the cattle system yielded 38 bu/acre more than the arable systems.
- The manured fields yielded 22 bu/acre more than the not manured.
- The 17.461 yielded 28 bu/a more than the FOS8500 in the arable system without manure which was 37% more for the standardized comparisons.
- The 17.461 yielded 24 bu/a less than the FOS8500 in the arable system with manure which was 15% less for the standardized comparisons.
- The 17.461 yielded 5 bu/a less than the FOS8500 in the cattle system without manure which was 4% less for the standardized comparisons.
- The 17.461 yielded 16 bu/a less than the FOS8500 in the cattle system with manure which was 8% less for the standardized comparisons.

Table 4. Analysis of variance for yield trials on 14 sites comparing 17.461 and FOS8500.

		bushels/acre			yield as % of FOS8500		
Source	**DF**	**SS**	**F Ratio**	**Prob > F**	**SS**	**F Ratio**	**Prob > F**
system	1	11110	3.1427	0.0915	459	3.2371	**0.0871**
hybrid	1	179	0.0506	0.8243	39	0.2728	0.6072
manuring	1	2906	0.822	0.3754	1255	8.859	**0.0075**
manuring*hybrid	1	1719	0.4864	0.4936	1255	8.859	**0.0075**
system*hybrid	1	326	0.0922	0.7645	459	3.2371	**0.0871**
system*manuring	1	1119	0.3167	0.5799	910	6.4231	**0.0197**
manuring*hybrid*system	1	565	0.1597	0.6936	910	6.4231	**0.0197**

The yield data from strip trials are portrayed graphically in Diagram 1. Simple inspection suggests that system is generally a major factor with the cattle system out-yielding the arable system. Furthermore, manuring appears to be a major factor for the 17.461 with manured corn especially yielding lower in the arable system.

Diagram 1. Yields of 17.461 and FOS8500 across 14 sites over two years.

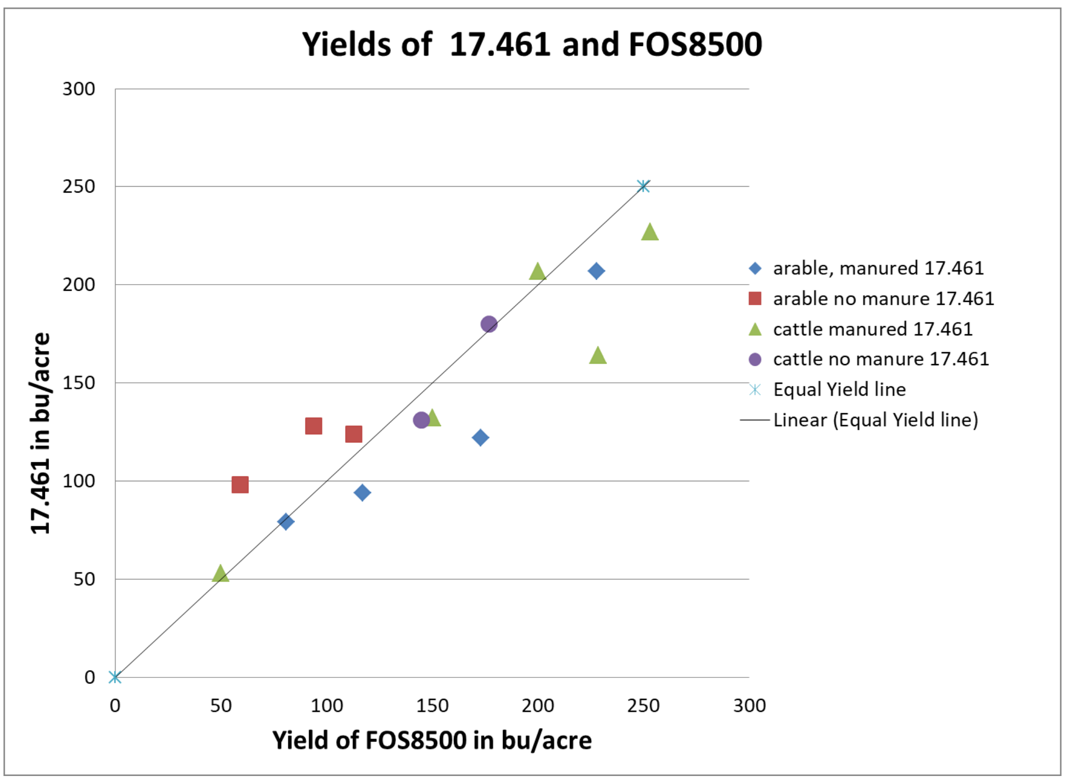


Table 5. Yields of 17.461 and FOS8500 from 14 sites.

Level	no sites	% of FOS check		bu/acre	
system effect					
arable	14	106	a	120	a
cattle	14	97	a	162	a
hybrid effect					
17.461	14	102	a	139	a
FOS8500	14	100	a	144	a
manure effect					
none	10	**108**	a	130	a
manured	18	**94**	b	152	a
system x hybrid interaction					
arable, 17.461	7	**111**	**a**	121	a
arable,FOS8500	7	**100**	**b**	119	a
cattle,17.461	7	**94**	**b**	156	a
cattle,FOS8500	7	**100**	**b**	169	a
manure x hybrid interaction					
none,17.461	5	**117**	a	136	a
none,FOS8500	5	**100**	b	125	a
manured,17.461	9	88	b	141	a
manured,FOS8500	9	100	b	163	a
manure x hybrid x system interaction					
none,17.461,arable	3	**137**	a	117	a
none,FOS8500,arable	3	**100**	b	89	a
none,17.461,cattle	2	96	b	156	a
none,FOS8500,cattle	2	100	b	161	a
manured,17.461,arable	4	85	b	126	a
manured,FOS8500,arable	4	100	b	150	a
manured,17.461,cattle	5	92	b	157	a
manured,FOS8500,cattle	5	100	b	176	a

Yield trials, paired manured and manured strip trials.

A second set of experiments was carried out for 2019 to 2021 on five sites where different Mandaamin hybrids were planted across manured and not manured plots. The hybrids grown on the plots included FOS8500, five hybrids with a C4-6 parent, and one (2019) or two hybrids (2020 and 2021) with NG10 as one of the parents. Because identity of the hybrids varied from year to year we averaged the yields of the C4-6 group of hybrids on each site and the NG10 group of hybrids on each site and compared them with the FOS8500 in an analysis of variance.

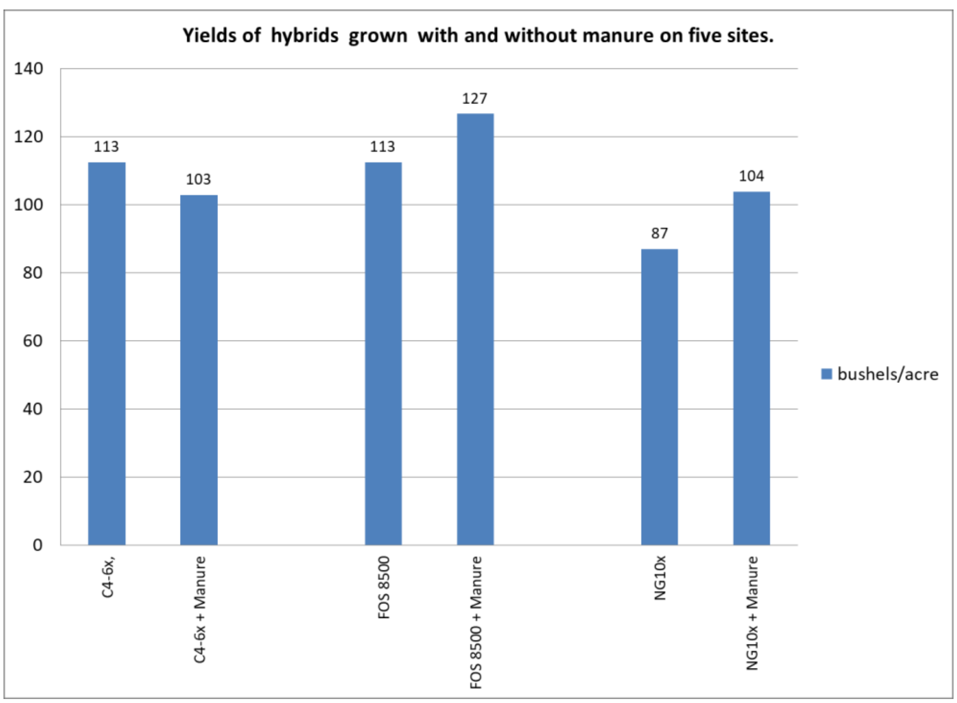
The analysis of variance is shown in Table 6. The only significant effect was of system.

Table 6. Analysis of variance for five different experiments comparing FOS8500, NG10, and C46 based hybrids.

Source	DF	SS	F Ratio	Prob > F
hybrid	2	2816	1.1162	0.3492
system	1	5610	4.4481	0.0492
manuring	1	363	0.2877	0.5982
system*manuring	1	114	0.0903	0.7673
hybrid*system	2	2553	1.0121	0.3832
hybrid*manuring	2	1022	0.4053	0.6727
hybrid*system*manuring	2	3	0.0012	0.9988

Irrespective of lack of statistical significance, the averages showed the same tendency as the experiment previously described. The arable system (Beiler Farm) yielded 94 bushels/acre while the cattle systems yielded 122 bu/acre. The manured plots yielded 114 bu/acre while the not manured yielded 107. Fos 8500 yielded 125 bu/axre while the C46 hybrids yielded 109 and the NG10 hybrids yielded 97 bu/acre. Diagram 2 shows the interaction between manuring and hybrids. Again manure appeared to reduce yields of the C4-6 hybrids while increasing yields of the FOS8500 and the NG10 hybrids.

Diagram 2. Interaction between manuring and hybrids on five sites.



Why does manure have a negative effect on hybrids that have C4-6 (461) as a parent?

An open enigma is why the negative effect on C4-6 hybrids, and why is it seemingly worse in its effect on lower yielding sites? We analyzed the mineral contents of the Beiler unreplicated strip trial which was on a lower organic matter upland soil. Table 7 shows that manuring decreased grain yields 20%, nitrogen uptake 32%, uptake of macronutrients 20-22%, and especially total uptake of micronutrients 46%) across the 461 hybrids.   Hybrids that respond positively to manuring had 38% more yield, 3% more N uptake, 8-16% more uptake of macronutrients, but 19% less micronutrient uptake. They had a positive response to manure or they had a lesser negative response. Most hybrids responded to manuring with some degree of decrease in uptake of micronutrients but it was much larger for the 461 hybrids. We assume this extreme reaction of the hybrids with C4-6 as a parent has to do with disruption of rhizophagy by the manure.

Table 7. Strip trial on the Beiler farm, 2020, showing response to manure for different hybrids. –M and +M indicate not manured and manured plots.

	Grain yield in bushels/acre			total Nitrogen uptake lbs/acre			K + Mg + Ca lbs/acre			P + S in lbs/acre			Cu + Fe + Mn + Zn in lbs/acre		
Hybrids with a positive manure response.															
hybrid	**-M**	**-M**	**%dif.**	-M	-M	%dif.	-M	-M	dif.	-M	-M	%dif.	-M	-M	%dif.
FOS 8500	**83**	**117**	**41**	67	67	**0**	108	91	**-16**	46	44	**-4**	3.75	2.42	**-35**
924.NG10	**68**	**105**	**54**	72	93	**29**	66	87	**31**	36	50	**38**	1.05	1.09	**0**
K5N.NG10	**53**	**77**	**45**	110	71	**-36**	78	59	**-24**	45	39	**-14**	2.27	0.80	**-65**
15.461	**65**	**94**	**45**	58	66	**13**	68	104	**52**	35	53	**53**	1.65	1.24	**-25**
17.2B24	**118**	**121**	**3**	95	101	**6**	124	121	**-2**	50	55	**9**	2.07	2.67	**29**
average	**77**	**103**	**38**	**81**	**80**	**3**	**89**	**92**	**8**	**42**	**48**	**16**	**2.16**	**1.64**	**-19**
Hybrids with a negative manure response.															
hybrid	**-M**	**-M**	**%dif.**	-M	-M	%dif.	-M	-M	%dif.	-M	-M	%dif.	-M	-M	%dif.
9215.461	**100**	**98**	**-2**	92	68	**-26**	99	57	**-42**	48	36	**-26**	2.27	0.58	**-74**
9211.461	**90**	**71**	**-21**	75	58	**-23**	99	76	**-23**	47	36	**-23**	2.02	1.13	**-44**
17.461	**128**	**94**	**-27**	118	78	**-34**	161	164	**2**	66	66	**0**	3.50	3.13	**-10**
UR65.461	**127**	**91**	**-28**	137	77	**-44**	161	119	**-26**	81	55	**-32**	3.98	1.87	**-53**
Average	**111**	**89**	**-20**	106	70	-32	130	104	-22	61	48	-20	2.94	1.68	**-46**

On the Weiss farm, which had a high fertility soils with a very high soil protein content, also sponsored hybrid trials with and without manure in 2019. Results shown in Table 8 are that 17.461 did respond negatively to manuring with a 19% reduction in grain yield and a 6% reduction in N uptake. However the effect of manure was positive for uptake of other nutrients. The differences between the response on the two sites could relate to the necessity for hybrids like 17.461 to exercise rhizophagy on soils with lower nutrient availability, and that they are prevented from doing that when manure is applied. This is not as large an issue where nutrients are more available such as they were on the Weiss farm in 2019. See section below on varietal trials in 2019.

Table 8. Strip trial on the Weiss/Bauer farm, 2019, showing response to manure for different hybrids. –M and +M indicate not manured and manured plots.

Hybrid	Soil Protein mg/kg			Grain yield in bushels/acre			total Nitrogen uptake lbs/acre			K + Mg + Ca lbs/acre			P + S in lbs/acre			Cu + Fe + Mn + Zn in lbs/acre		
	-M	+M	%dif.	-M	+M	%dif.	-M	+M	%dif.	-M	+M	%dif.	-M	+M	%dif.	-M	+M	%dif.
FOS 8500	9.25	10.7	15	177	214	21	196	239	22	300	189	-37	73	67	-9	3.51	4.71	34
17.2B24	10.3	11.4	11	167	172	3	213	280	31	279	285	2	66	86	30	4.18	5.04	21
C2B21.461	9.4	10.4	10	181	169	-7	223	241	8	323	244	-25	72	78	8	3.60	5.00	39
17.461	10.2	11.9	16	180	145	-19	237	222	-6	290	319	10	69	98	42	4.27	5.32	25

Results Varietal Trials 2019.

Soil test results. Table 9 shows soil test results from samples taken in early June from recently planted corn plots on all the farms except for the Weiss/Bauer fields, which was not analyzed at that time. Nutrient levels varied with high levels of calcium for the Adsit, Beiler, Anon 1 and 2 farms. Inspection of the two columns on the right hand side of the table reveals large difference between the two management approaches. On average, the farms with arable cash cropping based on inputs of chicken manure had greater levels of phosphorus and potassium. There was little difference in amounts of available N in June for the corn plants. However, the cattle-based operations had greater stocks of organic matter, carbon, and nitrogen and a higher C/N ratio. This included more particulate organic matter and greater stocks of potentially mineralizable nitrogen.

Table 9. Soil analysis results for 2019 varietal trials.

Soil	Arable Cropping poultry manure						dairy/beef-based farming with arable cropping								ave. Cash	ave. cow
Parameter	Adsit		Beiler		Doudlah		Anon1		Anon2		Zinniker		Anibas			
	ave.	s.e.	ave.	s.e.	ave.	s.e.	ave.	s.e.	ave.	s.e.	ave.	s.e.	ave.	s.e.		
NH4+ (mg/kg)	**3.0**	0.2	**2.8**	0.1	**1.8**	0.4	**3.1**	0.2	**4.0**	0.3	**5.5**	1.8	**4.1**	1.2	2.5	4.2
NO3- (mg/kg)	**22.3**	0.8	**20.9**	0.4	**22.1**	1.4	**20.2**	0.4	**20.8**	0.6	**21.2**	1.2	**24.5**	1.7	21.8	21.7
Inorganic N (mg/kg)	**25.2**	0.8	**23.7**	0.5	**23.9**	1.4	**23.3**	0.5	**24.8**	0.8	**26.7**	2.6	**28.5**	2.4	24.3	25.8
PMN (mg/kg)	**13.6**	2.8	**35.8**	3.9	**27.0**	2.1	**52.6**	2.2	**54.7**	2.7	**55.4**	2.6	**49.8**	1.8	25.5	53.1
pH	**6.2**	0.1	**7.2**	0.0	**6.9**	0.1	**6.7**	0.1	**6.6**	0.1	**6.7**	0.1	**7.2**	0.0	6.8	6.8
Bray I P (ppm)	**12**	1	**71**	5	**102**	9	**36**	11	**16**	3	**13**	3	**34**	3	61.9	24.5
K* (mg/kg)	**163**	6	**265**	26	**251**	9	**187**	19	**178**	15	**131**	17	**104**	4	226	150
Ca* (mg/kg)	**2755**	59	**3213**	51	**1568**	49	**3138**	87	**2976**	103	**1816**	73	**1835**	33	2512	2441
Mg* (mg/kg)	**792**	26	**1126**	13	**337**	13	**864**	32	**872**	41	**519**	26	**424**	10	752	670
TON (g/kg soil)	**1.2**	0.0	**1.3**	0.0	**0.9**	0.1	**2.0**	0.1	**2.0**	0.1	**1.4**	0.1	**1.4**	0.0	1.2	1.7
TOC (g/kg soil)	**10.4**	0.4	**12.4**	0.5	**8.8**	0.7	**21.1**	0.6	**21.5**	0.7	**14.0**	0.9	**14.4**	0.5	10.5	17.7
C/N ratio	**8.6**	0.2	**9.2**	0.1	**9.7**	0.3	**10.7**	0.1	**11.0**	0.1	**10.1**	0.2	**10.2**	0.1	9.2	10.5
POM-C (g/kg soil)	**1.3**	0.1	**2.3**	0.2	**1.5**	0.1	**2.9**	0.3	**3.1**	0.3	**2.8**	0.2	**3.3**	0.2	1.7	3.0

PMN means potentially mineralizable N. TOC and TON mean total organic carbon and N.

POM-C means particulate organic matter carbon.

To test significance of difference the three arable organic farms were compared with values for the three organic farms with cattle (Table 10). There was no difference in the amount of total inorganic N available in the spring. The arable organic had 157% more Bray P, 63% more K, 12% more Ca,and 25% more Mg than did the organic cattle sites. However, they also had only 73% as much Total N, 64% as much total C, 89% as high a level of C/N and 54% as much particulate organic matter C.

Table 10. Comparison of arable organic and cattle organic systems in 2019 varietal trials.

Parameter	scale	Arable Organic	Cattle Organic	p level	Arable/ cattle as %
NH_4^+	mg/kg	2.5	4.4	0.05	57
NO_3^-	mg/kg	21.8	22.1	0.84	99
total inorganic N	mg/kg	24.3	26.4	0.20	92
pH		6.8	6.9	0.83	99
Bray I P	ppm	61.9	24.1	0.24	257
K^+	mg/kg	226.5	139.2	0.09	163
Ca^{2+}	mg/kg	2511.8	2235.8	0.69	112
Mg^{2+}	mg/kg	751.7	603.4	0.61	125
total organic N	g/kg	1.2	1.6	0.13	73
total organic C	g/kg	10.5	16.6	0.08	64
C/N		9.2	10.4	0.04	89
particulate organic matter-C	g/kg	1.7	3.1	0.01	54
potentially mineralizable N	mg/kg	25.5	53.0	0.01	48

We analyzed crop performance parameters with system, hybrid, hybrid x system, and total organic N and C/N as covariates. The percentages of the total variance for performance that were accounted for by soil organic N plus + C/N were 36% for grain yield, 57% for stalks/acre, 51% for roots/acre, 45% for N uptake/acre, 66% for C uptake/acre, 45% for tissue %N, 87% for $\delta^{15}N$ in grain and 27% for $\delta^{15}N$ in roots.

Relationship between spring tests before planting and soil quality tests before harvest, varietal trials, 2019:

The relationship between the three farming systems and the soil test values are shown in Table 11. Obviously the use of cattle slurry on the conventional farm had boosted the soil protein levels beyond those in the other systems. The lower aggregate stability and higher bulk density on this farm was probably related to a greater content of sand in the soil composition. The percentage relationship between the arable and cattle based organic systems for the different fall measured parameters paralleled the results for organic matter related parameters in in the spring samples.

Table 11. Relationship between farming systems and September soil test values in 2019.

Parameter	scale	Arable Organic	Cattle Organic	Cattle Conv. Monoc.	differences p level	Arable/cattle as %
Protein	mg/kg	5.0c	7.3b	10.4a	0.0001	68
Protein Score	mg/kg	32.0c	58.8b	87.1a	0.0001	54
CO2 corr. BD	mg/kg	72.2b	89.3a	65.5b	0.0053	81

SLAN	ppm	82.0b	129.0a	119.7a	0.0001	64
Aggregate Stability	%	24.1b	29.0a	6.4c	0.0001	83
NO_3^- N	ppm	14.5c	30.6a	21.7b	0.0001	48
Bulk density	g/cm3	1.11b	1.00c	1.19a	0.0001	110

Relationships between the most pertinent parameters for the spring and fall sampling are shown in Table 12. These linear model analyses used total organic nitrogen and carbon (TON and TOC), C/N ratio, particulate organic matter carbon (POM)-C., and potentially mineralizable N (PMN) as determinant factors and protein, protein score, labile amino N (SLAN), nitrate, aggregate stability, bulk density, and CO2 respiration as respondent factors. Using the former factors as x factors explained the majority of the variation in the nitrogen related soil quality tests. In fact, the vast majority of the variation for the nitrogen related parameters could be explained by total carbon and nitrogen and the relationship between them (99.6% for soil protein, 99.7% for protein score; 96% for SLAN; 75% for nitrate, and even 85% for CO2 respiration). Soil C, N, and C/N attributed less to the variation in aggregate stability (4.1%) and bulk density (12.1%) but there, the young fractions of particulate organic matter C and potentially mineralizable N were important.

Table 12. Relationships between spring and fall soil tests in 2019.

	Protein		Protein score		SLAN		Nitrate		Aggregate Stability		Bulk Density		CO2 Respiration	
Parameter & variance	% of total SS	P level	% of total SS	P level	% of total SS	P level	% of total SS	P level	% of total SS	P level	% of total SS	P level	% of total SS	P level
PMN	0.0	0.9144	0.1	0.7664	4.0	0.4496	17.6	0.0052	48.0	0.0896	29.5	<.0001	15.1	0.2442
TON	35.3	<.0001	35.4	<.0001	39.5	0.0198	27.6	0.0006	1.2	0.7882	5.3	0.0663	35.1	0.0784
TOC	33.7	<.0001	33.8	<.0001	31.5	0.0363	27.4	0.0006	0.5	0.8626	3.9	0.1163	35.8	0.0753
C/N	30.6	<.0001	30.5	<.0001	24.8	0.0623	20.2	0.0029	2.4	0.6975	2.9	0.1695	13.8	0.2641
POM	0.3	0.4816	0.2	0.5517	0.2	0.8498	7.1	0.0702	48.0	0.0896	58.3	<.0001	0.2	0.9052
%N,C,C/N	99.6		99.7		95.8		75.3		4.1		12.1		84.8	
R2 Model%	85	<.0001	86	<.0001	77	<.0001	67	<.0001	39	<.0001	73	<.0001	23	0.0211

Following analysis of the data that had been condensed into types it became clear that there were significant differences between the arable organic, cattle based organic and conventional manured monoculture for every parameter measured. There were significant effects of farming systems on soil protein, protein core, CO2 respiration, SLAN, Aggregate stability, and bulk density (<0.0001) (Table 13). Regarding effects of systems and hybrids on the soil, contrasts showed that soil under 17.461 and 17.2B24 had higher aggregate stability but lower SLAN and nitrate production than FOS8500.

Table 13. Effects of hybrids on soil characteristics in 2019 varietal trials.

hybrid averages	SLAN	Nitrate	Aggregate Stability
	mg/l	ppm	Vol%
17.C2B2-4	106.7	18.8	22.0
17.461	109.0	20.0	21.1
C2B2-2.461	108.6	24.5	18.1
FOS8500	116.7	25.7	18.1
Contrasts	level of p		
17.C2B24:FOS8500	0.1010	0.0178	0.0363
17.461:FOS8500	0.2070	0.0475	0.1092
C2B2.C46:FOS8500	0.1828	0.6633	0.9819
both 17's:FOS8500	0.0956	0.0129	0.0340

Hybrid performance: The average yields of the different varieties are shown in Tables 14 and 15.

Table 14. Overall yields from varietal trials in 2019

Hybrid Combination 2019 strip trials.										
Farm	system	**FOS8500**	**15.C4-6**	**17.461**	**17.2B24**	**C2B2-1.4-6**	**C2B24-7.C46**	**C46.9.2)-11**	**NG10-2-3-2.Md1**	**average yield**
		bushels/acre								
Adsit	cash	81	60	79	112	70	65	16	11	**62**
Beiler	cash	173	112	122	103	139	148	154	152	**138**
Doudlah	cash	59	141	98	122	95	105	148	102	**109**
Anibas	dairy	150	154	132	131	123	115	105	127	**130**
Anon1	dairy	253	194	227	215	185	204	140	169	**198**
Anon2	dairy	228	161	164	188	151	209	92	191	**173**
Weiss/Bauer	dairy	214	161	145	172	169	124	144	170	**162**
Weiss/Bauer	dairy	177	204	180	167	181	130	138	116	**162**
Zinniker	beef	200	177	207	171	172	180	158		**181**
ave		**170**	**152**	**151**	**153**	**143**	**142**	**122**	**130**	**145**
Plots in blue were not fertilized (Doudlah), or fertilized with lower level of nutrients (Weiss/Bauer).										

Initial observations were 1) the commercial check gave the highest overall yield; 2) that the cattle-based systems had higher grain yields; 3) manure application at the Weiss farm did not appear to affect overall yields; 4) some varieties, especially the check, gave lower yields where they were not manured.

Table 15. Yield results for manured and not manured trials and for arable cash and cattle based trials in 2019.

Hybrid Combination 2019 strip trials.										
Farm		FOS8500	15.C4-6	17.461	17.2B24	C2B2-1.4-6	C2B24-7.C46	C46.9.2)-11	NG10-2-3-2.Md1	average yield
		bushels/acre								
Ave with manure		185	146	154	156	144	149	116	137	**149**
Ave. without manure		118	172	139	145	138	118	143	109	**135**
Ave cash		104	104	100	112	101	106	106	89	**103**
Ave cow		203	175	176	174	164	160	130	155	**168**

On average, the best producer on the cash system farms or under unmanured conditions was 17.2B24. . In the cases where manuring was omitted (Doudlah and Weiss notM) the yields of FOS8500 dropped strongly relative to the Mandaamin hybrids and in the case of Weiss, relative to the side by side manured plots.

Detailed research, varietal trials, 2019: As was referred to above, in order to elucidate relationships between farms, soils, and nitrogen efficiency/fixation, we focused research on five farms (Beiler, Doudlah, Anon, Weiss/Bauer, and Zinniker). Weiss/Bauer had two sites one with starter fertilizer, another with starter fertilizer and manure. That site had a history of manure slurry applications from the Weiss dairy operation. Farms were grouped according to farm type as described above. Anon had two replicated sites with different planting dates which were averaged. We studied plant performance, nutrient and N relationships and soil on a subset of four hybrids. The four hybrids were described above as the FOS8500 control, 17.461, 17.2B24, and C2B2-1.C46. Data was analyzed using type of farm, hybrid, and type x hybrid as sources of variation in an analysis of variance.

Before describing detailed statistics, the general patterns in relationships between the different parameters in magnitude are shown in Table 16. Here, results for the different hybrids are presented as a percentage of the FOS8500 checks in each system. Values in red indicate values that are 5% or more below the check value. Values for accumulated elements were calculated as an average % difference for the ratio of Mandaamin hybrid/FOS8500 across measurements of Al, Cu, Fe, Mn, Si, Sr, Ti, Zn for the microelements and of C, N, Ca, Mg, K, P, and S for the macro-elements.

Averaged across the systems, Mandaamin hybrids had lower (10-11% grain yields), greater N accumulation (3-9%), greater accumulation of macroelements (14 to 20%); greater accumulation of microelements (5-19%), greater accumulation of dry matter and N in stalks, and in part in roots. The hybrid C2B2-1.C46 differed from the other Mandaamin hybrids by having lower stalk production and macroelement uptake.

Table 16. Grain yield and mineral uptake as affected by hybrid and farming systems in 2019.

Farming System	Hybrid	Grain DM	Grain N	Stalk N	Root N	Total N	Micronutrient uptake	Macronutrient uptake
		values as % of FOS8500						
arable crop organic	17.461	**95**	**94**	**166**	**42**	**107**	**97**	**109**
arable crop organic	17.2B24	**97**	**97**	**187**	**67**	**115**	**115**	**108**
arable crop organic	C2B2-1.C46	**101**	**101**	**174**	**83**	**116**	**123**	**107**
arable crop organic	FOS8500	**100**	**100**	**100**	**100**	**100**	**100**	**100**
cattle based organic	17.461	**91**	**105**	**143**	**110**	**112**	**134**	**123**
cattle based organic	17.2B24	**85**	**86**	**150**	**98**	**98**	**136**	**114**
cattle based organic	C2B2-1.C46	**77**	**80**	**103**	**94**	**85**	**112**	**96**
cattle based organic	FOS8500	**100**	**100**	**100**	**100**	**100**	**100**	**100**
cattle mono corn conv	17.461	**83**	**98**	**126**	**97**	**106**	**115**	**126**
cattle mono corn conv	17.2B24	**87**	**92**	**122**	**241**	**114**	**110**	**125**
cattle mono corn conv	C2B2-1.C46	**90**	**106**	**82**	**203**	**107**	**107**	**110**
cattle mono corn conv	FOS8500	**100**	**100**	**100**	**100**	**100**	**100**	**100**
average across systems	17.461	**90**	**99**	**145**	**83**	**108**	**115**	**119**
average across systems	17.2B24	**89**	**92**	**153**	**135**	**109**	**120**	**116**
average across systems	C2B2-1.C46	**89**	**96**	**120**	**127**	**103**	**114**	**105**
average across systems	FOS8500	**100**	**100**	**100**	**100**	**100**	**100**	**100**

For each parameter analysis of variance revealed significant differences due to plant part, but not to hybrid, pedigree, farm x hybrid, or part x hybrid[2]. Yields of carbon, nitrogen, and dry matter are shown in Table 17 including information on significance of differences. The arable farming systems had the lowest total dry matter yield and lowest yield of grain, stalks, and roots. The highest grain yields and the highest partition of N into grain were achieved in the organic cattle based systems, but the monoculture corn system achieved the highest stalk and N yields.

The FOS8500 had significantly higher harvest index which paralleled higher dry matter accumulation in grain and percent of total plant N in grain than did the two hybrids with 17 as a parent (17.2B24 and 17.461). However the latter two hybrids had greater total N uptake, dry matter in stalks, and % N in grain. 17.2B24 had the most C and N uptake. However, the two hybrids with C2B2 as a parent also had more total C, N, and dry matter in their roots than did 17.461 and FOS8500.

Table 17. Grain, stalk (stover) and root yields, harvest index (HI), total N, and the percentage of total N in grain for hybrid variety trials in 2019.

[2] Throughout this report there is a significant difference between means at p=5% if the means within a parameter do not share the same letter after the mean values.

	grain	grain	stalks	roots	total dm	HI	total N	N in grain
Farming System	bu/acre	lbs dm/a				%	lbs/a	%
Arable organic/poultry	114c	5,388c	4,072c	830.4c	10,291c	56b	92c	62b
Organic cattle based	195a	9,202a	4,646b	985b	14,833b	67a	173b	73a
Conv. Mono. Cattle	176b	8,303b	6,143a	2597a	17,054a	57b	231a	58b
Hybrid	bu/a	lbs dm/a				HI	lbs/a	%
17.2B24	156 b	7,384b	5,268a	1,759a	14,411a	58b	172a	60b
17.461	158b	7,471b	5,491a	1,118b	14,080a	57b	172a	63ab
C2B2.C46	156b	7,290b	4,626b	1,759a	13,675a	61a	159a	65ab
FOS8500	177a	8,389a	4,430b	1,248b	14,057a	64a	159a	69a
Mandaamin vs FOS in %	89	89	112	124	100	92	105	91
Contrast	level of p							
two 17's and FOS8500	**0.0038**	**0.0038**	**0.0001**	0.2667	0.72	**0.0001**	0.3914	**0.0219**

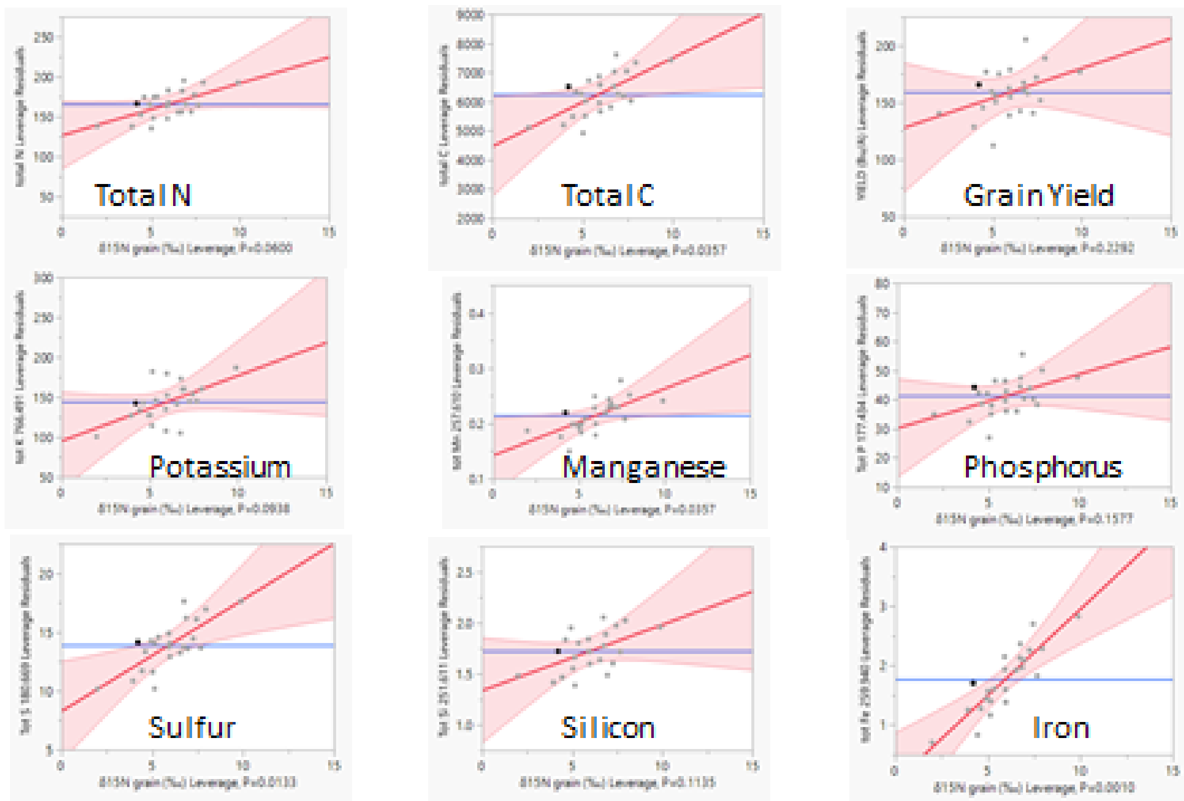
An analysis was made to model to which extent uptake of minerals was influenced by covariates consisting of the $\delta^{15}N$ values for grain, stalks, and roots. Only $\delta^{15}N$ for grain showed significant effects and those relationships were all positive relationships (Table 18 and Diagram 3).

Table 18 shows results of analysis of variance examining effects of covariates on nutrient uptake per acre.

		N	C	Al 237	Ca	Cu	Fe	K	Mg	Mn	P 177	S 180	Si	Sr	Ti	Zn
Source	DF	Prob > F														
Pedigree	3	0.7375	0.9183	0.7177	**0.0087**	0.9319	0.1455	0.5503	0.3698	0.4522	0.712	0.5128	**0.0476**	**0.0353**	**0.0162**	0.5401
system	2	**0.0109**	**0.0225**	0.2842	0.577	0.4194	**0.0623**	**0.0005**	0.8198	**0.0043**	**0.0091**	**0.007**	0.905	**0.012**	**0.0052**	0.531
system*Pedigre	6	0.7671	0.7579	0.9935	0.7088	0.7465	0.1905	0.667	0.8929	0.1653	0.9026	0.3857	0.3805	0.5669	0.2993	0.9583
δ15N grain (‰)	1	**0.06**	**0.0357**	0.1092	0.2515	0.4025	**0.001**	**0.0938**	0.3084	**0.0357**	0.1577	**0.0133**	0.1135	**0.0222**	**0.0002**	0.4578
δ15N root (‰)	1	0.7931	0.7978	0.6029	0.7694	0.7803	0.1252	0.4409	0.9514	0.4639	0.5586	0.7645	0.4974	0.3458	0.9799	0.5521
δ15N stalk (‰)	1	0.9101	0.9442	0.4	0.3283	0.4564	0.2935	0.6238	0.4699	0.9791	0.2557	0.7935	0.8493	0.4679	0.8714	0.6717
plants/acre	1	0.8399	0.1616	0.4334	0.4341	0.8369	0.1317	**0.0824**	0.434	**0.0926**	0.7906	**0.0974**	0.1937	0.1688	0.1474	0.9188
%SS Pedigree		5	2	14	74	6	12	4	42	6	5	6	48	30	21	37
%SS system		64	50	30	4	27	14	75	5	47	70	45	1	34	23	22
%SS Sys x Ped		12	13	6	12	47	20	7	24	25	8	17	29	11	9	21
%SS δ15N grain		18	25	33	5	11	43	6	14	13	9	23	12	17	44	10
%SS δ15N stalk		0	0	3	0	1	5	1	0	1	1	0	2	2	0	6
%SS δ15N root		0	0	8	3	8	2	0	7	0	6	0	0	1	0	3
%SS plants/a		0	9	7	2	1	5	7	8	7	0	8	8	5	3	0

Diagram 3. Relationship between δN^{15} and uptake of nutrients.

Relationship between the $\delta^{15}N$ isotope ratio in the grain and the total uptake of different minerals/acre.



Soil test values generally correlated poorly with both %N and $\delta^{15}N$ in tissues. On the other hand, both of these parameters seemed to correlate well with plant production and nutrient uptake parameters. The %N seemed to have a central place as a high level of correlation was found between %N and total C (R^2=62%), total N (R^2=78%), total dry matter (R^2=22%), and P (R^2-62%), S (R^2=69%), Cu (R^2=12%), and Zn (R^2=34%) uptake by plant parts. The $\delta^{15}N$ had similar significant correlation values with most of these parameters, but the R^2 values were lower than for %N. This indicates that the two kinds of measurements are linked. Approximately 60 to 69% of the N that was accumulated in the plant was found in the grain. But correlations between the $\delta^{15}N$ in the grain and the same performance and uptake factors showed little relationship.

Soil tests as explanatory factors for crop performance: Regression studies tested a model that combined soil protein, protein core, CO2 respiration, SLAN, aggregate stability, and bulk density for its relationship to crop performance parameters. Soil protein content, protein score, aggregate stability and bulk density proved significantly correlated with many crop performance parameters. The combination of these factors accounted for the vast majority of total sum of squares variation. There was little significant little or no significant statistical relationship between the other soil tests and crop performance parameters. Stalk yield related to bulk density and nitrate. The model tested accounted for less of the variation for %N and $\delta^{15}N$. There was no statistical significance for any of the individual tests for %N; and only aggregate stability (p=0.01) and bulk density (p=0.07) showed some level of significance in relationship to $\delta^{15}N$.

Table 19. Relationship between soil tests and crop performance for variety trials in 2019.

	grain yld	stlk yld	root yld	N yld	C yld	%N	delta15N	
	level of p							
protein	**0.06**	1.00	**0.05**	**0.10**	**0.01**	0.69	0.46	
protein score	**0.01**	0.27	0.21	**0.00**	**0.00**	0.17	0.21	
CO2 corr to BD	0.86	0.54	0.57	0.30	0.46	0.93	0.40	
SLAN ppm	0.23	0.87	0.93	0.29	0.47	0.66	0.86	
Aggregate Stability	0.77	0.07	0.16	**0.06**	**0.02**	0.52	**0.01**	
Nitrate N	0.47	**0.00**	0.86	0.83	0.35	0.94	0.52	
BD g/cc	**0.01**	**0.01**	0.90	**0.05**	**0.00**	0.64	**0.07**	
	grain yld	stlk yld	root yld	N yld	C yld	%N	delta15N	Average
	% of total SS							
protein	19	0	50	12	18	5	4	16
protein score	33	6	20	46	35	66	12	31
CO2 corr to BD	0	2	4	5	1	0	6	3
SLAN ppm	7	0	0	5	1	7	0	3
Aggregate Stability	0	16	25	16	17	14	49	20
Nitrate N	3	45	0	0	2	0	3	8
BD g/cc	37	32	0	16	25	7	25	20
R2 Model	0.75	0.72	0.57	0.90	0.83	0.73	0.40	
p level model	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0027	

Nitrogen efficiency and fixation in varietal trials, 2019:

Two isotopes are found in nature: ^{15}N and ^{14}N. Little ^{15}N is found, but for what there is it tends to accumulate more in soil organic matter than in air. The relative amount of ^{15}N is expressed by the $\delta^{15}N$ value which also indicates the ratio between the two natural isotopes. As the values increase, there is more ^{15}N. Air is richer in ^{14}N than soil. Nitrogen fixation from the air involves the accumulation of N2 from the atmosphere which dilutes the relative amount of ^{15}N and depresses the $\delta^{15}N$.

Diagram 4 shows that the Mandaamin hybrids had higher values of $\delta^{15}N$ in their roots and lower values in their grain than the commercial check. Increase in ^{15}N in roots is probably associated with uptake of ^{15}N rich easily extractable soil organic matter or microbial biomass. These results fit with our hypothesis that the Mandaamin hybrids increase uptake of N from microbial sources or their decomposition products and also fix N_2. To fit the data with appropriate calculations we made the following assumptions:

1) The ^{15}N content in the roots represented the isotope composition of N derived from the soil coupled with microbial biomass.
2) The difference in root $\delta^{15}N$ between the FOS8500 and the Mandaamin hybrids was due to the roots of the Mandaamin hybrids appropriating more N from microbial biomass or easily extratable organic matter.
3) The difference between the highest $\delta^{15}N$ achieved in the root or the stalk and the $\delta^{15}N$ found in the grain indicated the dilution effect due to N_2 fixation and the amount of N derived from the air (NDFA) in the grain.

Diagram 4. The $\delta^{15}N$ values for hybrids and their plant parts.

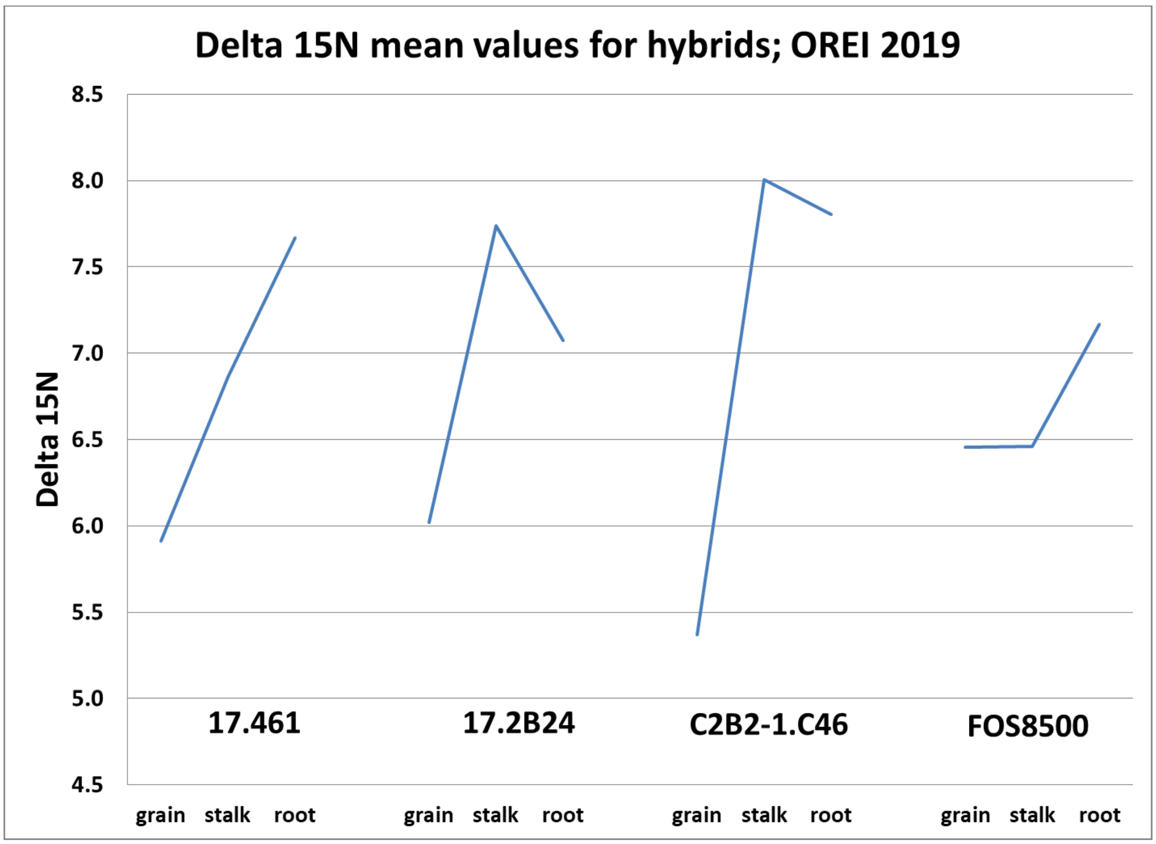


Diagram 5 shows the interaction between farming systems and $\delta^{15}N$ levels for different organs of the four hybrids. There was little difference between the roots, stalks and grain $\delta^{15}N$ levels for 17.461 on the arable organic sites, but substantial difference between it and the FOS8500 control. In that case the difference in the grain $\delta^{15}N$ levels between that hybrid and the control were used to calculate NDFA.

Table 20. Data for %N and $\delta^{15}N$ levels of hybrids grown in 2019.

		%N			delta 15N		
hybrid	Part	mean	stdev	cv	mean	stdev	cv
17.461	grain	1.44	0.29	0.20	5.94	1.99	0.33
17.461	stalk	0.91	0.21	0.23	6.75	2.58	0.38
17.461	root	0.71	0.22	0.31	8.00	2.16	0.27
17.2B24	grain	1.32	0.25	0.19	6.08	4.01	0.66
17.2B24	stalk	0.99	0.23	0.23	7.79	2.36	0.30
17.2B24	root	0.76	0.31	0.41	7.08	3.28	0.46
C2B2-1.C46	grain	1.36	0.30	0.22	5.28	2.88	**0.54**
C2B2-1.C46	stalk	0.83	0.09	0.11	7.89	3.74	0.47
C2B2-1.C46	root	0.72	0.35	0.49	7.32	4.89	0.67
FOS8500	grain	1.27	0.18	0.14	6.27	2.66	0.42
FOS8500	stalk	0.83	0.27	0.33	5.41	0.98	0.18
FOS8500	root	0.74	0.23	0.32	6.38	1.58	0.25

Diagram 5. The $\delta^{15}N$ values for hybrids and their plant parts grown in different farming systems.

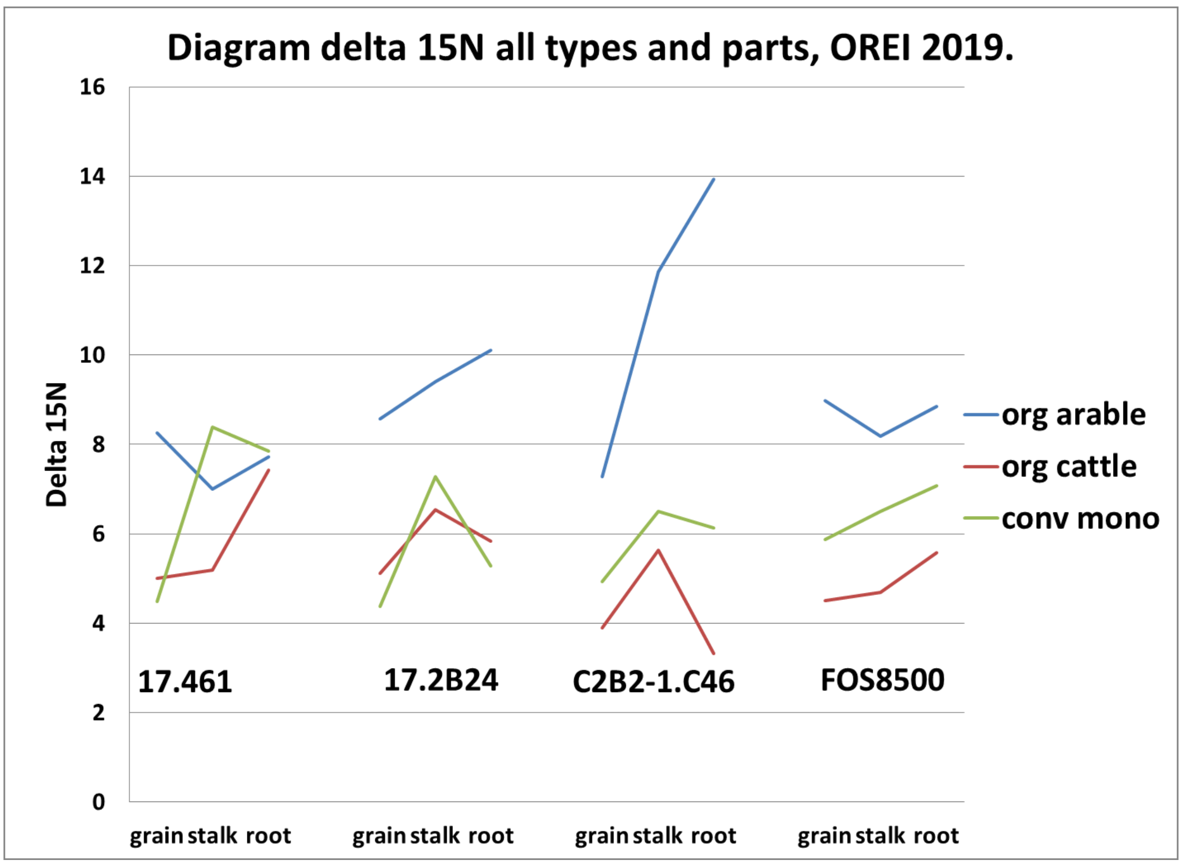


Table 21. Estimates of nitrogen derived from air (NDFA) and microbial biomass in 2019.

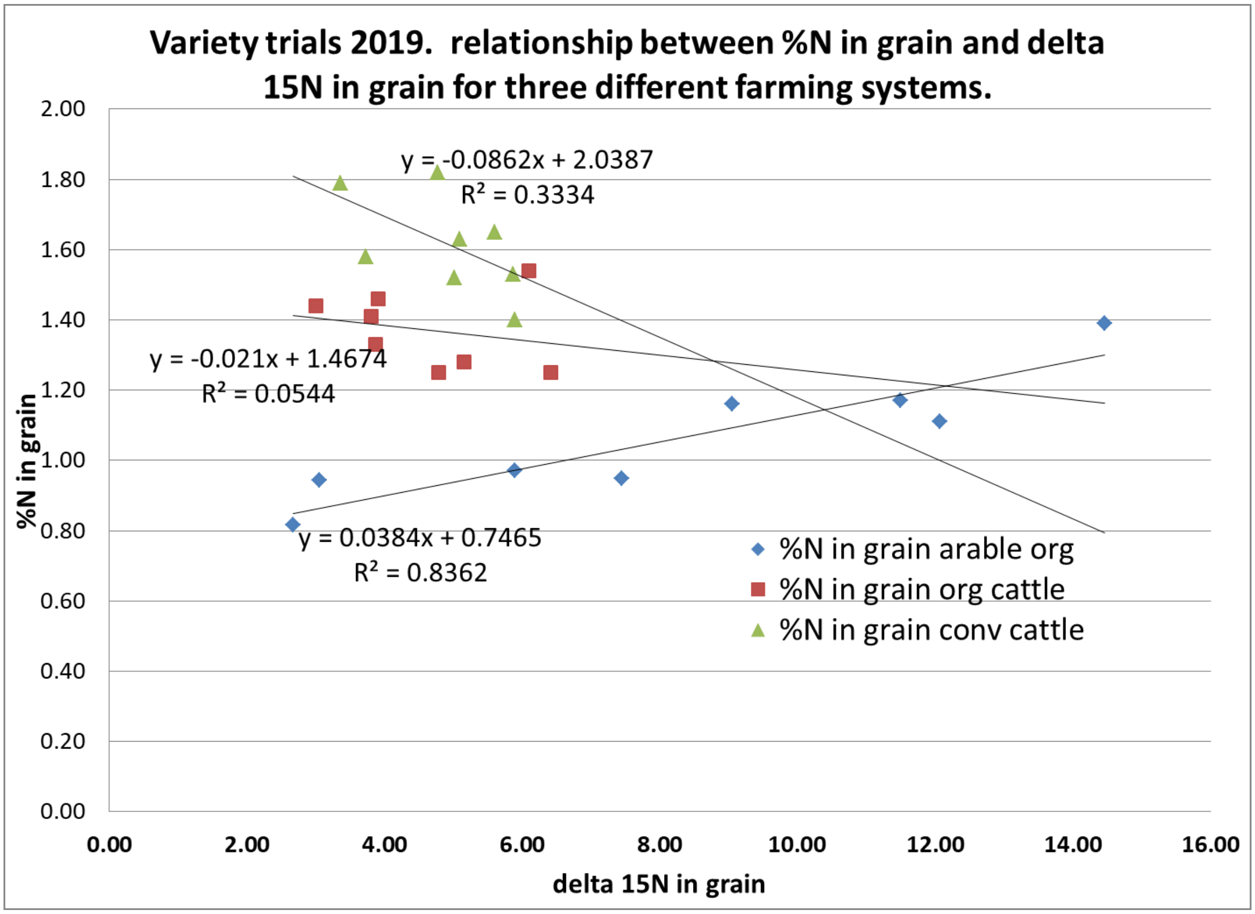
Hybrid	arable organic	cattle based organic	conv cattle mono	average
	best estimate %NDFA			
17.2B24	15	22	**40**	26
17.461	8	33	**47**	29
C2B2C46	**48**	31	24	34
FOS8500	-1	19	17	12
average	17	26	32	

	arable organic	cattle based organic	conv cattle mono	average
Hybrid	N from microbial biomass, available OM			
17.2B24	-15	-39	-12	-22
17.461	13	-33	-29	-16
C2B2C46	-58	-20	0	-26
FOS8500	0	0	0	0
ave Mand	-20	-31	-14	

The relationship between N uptake by the crop and the $\delta^{15}N$ ratios in grain also seemed to differ according to farming system (Diagram 6). The arable organic system had the highest $\delta^{15}N$ values which is congruent with the fact that the arable soils had the lowest C/N ratios. Soils with low C/N ratios have have microbial biomass with high $\delta^{15}N$ values (Craine et al., 2015). According to our calculations, the arable organic system had the lowest nitrogen derived from air values (17%), but medium estimates for N from microbial biomass (20%). This is assumed to have resulted in predominance of rhizophagy contributing loading N in the grain and a positive and significant linear relationship between $\delta^{15}N$ and %N in grain (R2=0.836, p = 0.0001 for intercept and 0.0015 for the $\delta^{15}N$ effect). On the other hand, corn grown in the conventional monoculture system averaged the highest N derived from air values (32%) but the lowest N derived from microbial/plant rhizophagy (14%). It showed a negative relationship between $\delta^{15}N$ and %N in grain (R2=0.3334, p = 0.0001 for intercept and 0.1339 for the $\delta^{15}N$ effect), suggesting indeed that N_2 fixation was contributing more to grain loading with N. On the other hand, the corn grown in the cattle based organic system had the second highest estimates for N derived from the air (26%) and the highest values for N derived from rhizophagy (31%). It showed no clear relationship between $\delta^{15}N$ and %N in grain (R2=0.0545, p = 0.0001 for intercept and 0.578 for the $\delta^{15}N$ effect). This implies that rhizophagy and N_2 fixation were both contributing to grain loading with N.

Similarly, a positive linear relationship was found between $\delta^{15}N$ and total N uptake for the arable organic system (R2 = 0.573, p = 0.067 for intercept and 0.0296 for the $\delta^{15}N$ effect. However there was no significant correlation for the conventional (R2 = 0.158) or the organic cattle system (R2 = 0.044).

Diagram 6. Relationship between %N and $\delta^{15}N$ in grain for different farming systems.



The hybrid 17.461 showed a strong negative linear relationship between δ^{15}N and %N (R^2=0.60, p = 0.0019 for intercept and 0.07 for the δ^{15}N effect) and a similar relationship between δ^{15}N and lbs of N/acre (R^2=0.534, p = 0.102 for intercept and 0.099 for the δ^{15}N effect). None of the other hybrids showed such relationships (R^2 values lower than 0.16).

Concentration of nutrients in varietal trials of 2019. Mineral analyses on the 2019 varietal trial revealed statistically significant effects of systems and plant parts on most of the minerals tested. The factors tested in the analysis that were associated with pedigree (pedigree, system x plant part, system x pedigree, system x plant part x pedigree) accounted for significant portions of the total variation for macronutrients P, S, and for micronutrients Al, Cu, Fe, Mn, Ti, and Zn. For macronutrients, 5 to 28% of the total sum of squares accounted for by the model was associated with pedigree. Pedigree affected variation more for micronutrients where 2 to 83% of the total variation was associated with pedigree.

Table 22. Analysis of variance for different macronutrients in plant tissues in 2019 hybrid trials.

Significance of analysis of variance factor for the concentration of different macronutrients							
		%N	Ca	K	Mg	P	S
Source of variation	Df	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
system	2	**<.0001**	**0.03**	**<.0001**	**0.00**	**<.0001**	**0.07**
plant part	2	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Pedigree	3	0.44	**0.04**	0.34	0.69	0.47	0.91
system x plant part	4	**0.10**	0.25	**0.00**	**0.00**	**0.00**	**0.07**
System x Pedigree	6	0.91	0.87	0.97	0.96	0.98	0.79
plant part x pedigree	6	0.64	**0.01**	0.31	0.88	**0.05**	0.39
system x plant part x pedigree	12	0.76	0.99	0.99	0.98	0.25	0.71
% variation associated with pedigree		5	5	6	6	13	28

Significance of analysis of variance factor for the concentration of different micronutrients									
		Al 237	Cu	Fe	Mn	Si	Sr	Ti	Zn
Source of variation	Df	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F	Prob > F
system	2	**0.01**	**0.08**	**0.02**	**<.0001**	**0.01**	0.14	**0.01**	0.56
plant part	2	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**0.00**
Pedigree	3	0.27	**0.04**	0.37	0.17	0.83	**0.01**	0.48	**0.00**
system x plant part	4	**0.00**	**0.00**	**0.00**	**<.0001**	**0.02**	0.57	**0.00**	0.25
System x Pedigree	6	0.93	**0.00**	0.73	0.12	0.88	0.86	0.77	**0.01**
plant part x pedigree	6	0.24	**0.00**	0.45	**0.10**	0.86	**0.00**	0.63	**0.00**
system x plant part x pedigree	12	0.96	**<.0001**	0.85	0.37	0.99	0.94	0.87	**0.00**
% variation associated with pedigree		18	64	24	11	2	3	13	83

The mean values for the plant part x pedigree interaction for macronutrients and microminerals are shown in Table 23. Also shown is the value averaged across all macronutrients or microminerals for the Mandaamin hybrids relative to the FOS8500 check.

On average the 17.461 had 14% and 13% higher macronutrient content in its grain and stalks, respectively, but 6% less in its roots than did the FOS8500. It had also 30% more and 6% less micronutrients in its grain and stalks, respectively, but 37% higher micronutrient content in its roots than did the FOS8500.

The C2B2.C46 had 12% higher and 3% lower macronutrient content in its grain and stalks relative to FOS8500. But it had equal amount of macronutrients in its roots as did the FOS8500. It had also 6% higher and 7% less micronutrients in its grain and stalks, respectively, but 67% higher micronutrient content in its roots than did the FOS8500.

The 17.2B24 had 6% and 12% higher macronutrient content in its grain and stalks relative to FOS8500. But it had 9% lower content of macronutrients in its roots as did the FOS8500. It had also 6% higher and 4% more micronutrients in its grain and stalks, respectively, but only a 1% difference in micronutrient content in its roots than did the FOS8500.

To summarize, the two hybrids with C4-6 in their parentage showed enhanced ability to accumulate macronutrients and micronutrients in their grain. This was especially the case for 17.461 which had 14% more macronutrients and 30% higher micronutrient content than the FOS8500. They also showed enhanced ability to accumulate micronutrients in their roots relative to the FOS8500 (37% and 67% more for the 17.461 and C2B2.C46, respectively).

Table 23. Concentration of minerals in hybrid tissue parts, hybrid trials 2019.

Part x hybrid	N	Ca	K	Mg	P 177	S 180	relative to FOS
	%	average in µg/g					%
Corn,17.2B24	1.33	37	4172	1122	3062	1038	106
Corn,17.461	1.43	42	4114	1244	3382	1082	114
Corn,C2B2-1.C46	1.38	36	4072	1233	3343	1192	112
Corn,FOS8500	1.27	35	3911	1073	2828	980	100
Stalk,17.2B24	1.00	3804	16168	2587	2872	954	112
Stalk,17.461	0.93	3614	17715	2649	3241	960	113
Stalk,C2B2-1.C46	0.85	2867	17146	2362	2442	800	97
Stalk,FOS8500	0.89	2715	16034	2249	3104	867	100
Root,17.2B24	0.77	1183	13610	1266	1089	718	91
Root,17.461	0.71	1136	18038	1199	1260	740	94
Root,C2B2-1.C46	0.75	1318	15279	1101	1784	737	100
Root,FOS8500	0.70	1134	20025	1263	1483	795	100
std error	0.06	169	1556	195	224	80	

Part x Hybrid	Al237	Cu	Fe	Mn	Si	Sr	Ti	Zn	relative to FOS
	average in µg/g								%
Corn,17.2B24	50	1.94	41	4.4	6	0.07	0.16	16.9	106
Corn,17.461	59	3.91	61	5.7	6	0.08	0.15	26.3	130
Corn,C2B2-1.C46	51	2.21	46	4.3	5	0.07	0.16	20.9	106
Corn,FOS8500	39	3.90	45	4.2	3	0.08	0.15	20.2	100
Stalk,17.2B24	88	7.88	193	35.6	334	7.18	2.06	29.0	104
Stalk,17.461	88	7.15	171	30.0	319	6.50	1.68	29.9	95
Stalk,C2B2-1.C46	80	7.07	196	31.0	290	5.73	1.84	26.2	93
Stalk,FOS8500	84	8.74	184	35.6	316	5.49	1.64	40.2	100
Root,17.2B24	294	3.81	405	17.5	124	2.56	7.18	5.2	101
Root,17.461	245	10.46	241	10.5	99	2.49	5.62	38.8	137
Root,C2B2-1.C46	490	9.09	608	24.0	128	2.89	9.52	25.0	167
Root,FOS8500	234	4.36	339	18.2	113	2.38	6.31	11.5	100
std error	58	1.07	89	2.6	21	0.21	1.13	4.0	

For macronutrient uptake, the differences between the average for the Mandaamin hybrids and the FOS8500 in nutrient concentration were greatest (8%) in the conventional system, intermediate (6%) in the organic cattle system, and least (2%) in the organic arable system. For micronutrient uptake, the differences between the Mandaamin hybrids and the FOS8500 were least (-5%) in the conventional system, and about the same (17% and 18%, respectively) in the organic cattle and the organic arable system. Hence enhanced micronutrient concentration for the Mandaamin hybrids was associated with the organic sites while enhanced macronutrient concentration was associated with the conventional sites.

Table 24. Concentration of mineral macronutrients in hybrids grown in variety trials in 2019.

system x pedigree	N	Ca	K	Mg	P 177	S 180	relative to FOS
	%	µg/g					%
arable organic,17.2B24	0.80	1518	11526	1479	2440	793	100
arable organic,17.461	0.74	1413	12525	1568	2756	902	104
arable organic,C2B2-1.C	0.80	1477	12145	1538	2561	850	103
arable organic,FOS8500	0.73	1281	13470	1565	2516	829	100
cattle organic,17.2B24	1.00	1926	8664	1948	1760	883	107
cattle organic,17.461	1.03	1837	10147	1984	1816	851	109
cattle organic,C2B2-1.C4	0.92	1554	8195	1876	1862	928	101
cattle organic,FOS8500	0.92	1424	9509	1790	1776	918	100
conv mono,17.2B24	1.30	1579	13760	1548	2823	1034	109
conv mono,17.461	1.30	1542	17195	1540	3312	1030	114
conv mono,C2B2-1.C46	1.26	1189	16157	1283	3146	951	102
conv mono,FOS8500	1.21	1180	16990	1228	3123	895	100
std error	0.06	169	1556	195	224	80	

system x pedigree	Al 237	Cu	Fe	Mn	Si	Sr	Ti	Zn	relative to FOS
	µg/g								%
arable organic,17.2B24	190	3.2	305	19.2	172	3.29	4.39	16.4	106
arable organic,17.461	171	10.0	177	16.4	126	3.02	2.37	42.3	119
arable organic,C2B2-1.C46	266	3.3	487	27.9	152	3.03	5.95	18.9	131
arable organic,FOS8500	143	4.1	252	23.7	152	2.91	3.31	22.6	100
cattle organic,17.2B24	177	6.1	228	26.3	171	3.17	3.62	17.8	114
cattle organic,17.461	139	6.1	191	18.0	166	2.79	3.46	20.7	102
cattle organic,C2B2-1.C46	273	9.9	245	21.1	161	2.80	3.95	31.6	136
cattle organic,FOS8500	143	5.5	199	21.7	166	2.40	3.27	19.4	100
conv mono,17.2B24	66	4.3	107	11.9	120	3.35	1.39	16.9	90
conv mono,17.461	82	5.4	103	11.7	131	3.27	1.62	32.0	103
conv mono,C2B2-1.C46	82	5.2	118	10.4	109	2.86	1.62	21.6	94
conv mono,FOS8500	71	7.4	117	12.7	115	2.65	1.52	29.9	100
std error	58	1.1	89	2.6	21	0.21	1.13	4.0	

Total nutrient uptake per acre, 2019 Varietal trials. The nutrient uptake by plants (total of root, stalk and grain) in the experiment is shown in Tables 25 and 26. Statistical analysis revealed few significant differences between hybrids for individual nutrients. But in the aggregate, the Mandaamin hybrids averaged the same C production per acre, but 13% more macronutrient uptake than the FOS8500. This was 7% more N, 44% more Ca, 6% more K, 15% more Mg, 2% more P, and 6% more S. The 17.461, 17.2B24, and C2B2.C46 averaged 19%, 16% and 5 % greater uptake of macronutrients per acre, respectively than did the FOS8500. Relative to the check the highest average uptake by the Mandaamin hybrids (21%) was in the conventional system, while the organic arable and organic cattle systems averaged 8% and 11% more than the check, respectively.

Table 25. Total uptake of macro nutrients in hybrid corn grown in different farming systems in 2019.

		Total C	total N	Ca	K	Mg	P177	S180
system	hybrid	lbs/acre						
arable crop organic	17.461	4500	90	5.0	34.1	5.8	10.8	3.1
arable crop organic	17.2B24	4645	97	5.4	34.1	5.5	9.4	2.9
arable crop organic	C2B2-1.C46	4592	98	4.3	34.7	5.6	10.1	3.3
arable crop organic	FOS8500	4500	84	3.9	33.6	5.6	10.3	2.9
cattle-based organic	17.461	7120	196	7.8	35.6	9.6	12.6	5.0
cattle-based organic	17.2B24	6609	172	7.5	34.1	8.6	11.7	4.6
cattle-based organic	C2B2-1.C46	5913	149	5.1	27.9	7.9	10.9	4.5
cattle-based organic	FOS8500	6872	175	4.3	33.0	7.3	11.8	5.1
cattle mono corn conv	17.461	7227	230	8.7	83.4	9.4	21.5	6.3
cattle mono corn conv	17.2B24	8025	247	9.1	75.4	9.6	18.8	6.6
cattle mono corn conv	C2B2-1.C46	7823	233	6.0	80.8	7.8	19.0	6.1
cattle mono corn conv	FOS8500	7392	217	5.5	68.9	7.1	18.0	5.3
average	17.461	6282	172	7.2	51.0	8.3	15.0	4.8
average	17.2B24	6426	172	7.3	47.9	7.9	13.3	4.7
average	C2B2-1.C46	6109	160	5.1	47.8	7.1	13.3	4.6
average	FOS8500	6254	159	4.5	45.2	6.7	13.4	4.4

Table 26. Total uptake of nutrients relative to the commercial check FOS8500 when grown in different farming systems in 2019.

		total C	total N	Ca	K	Mg	P177	S180	Average w/o C
system	hybrid	lbs/acre of macronutrients as % of FOS8500							
arable crop organic	17.461	100	**107**	**130**	102	104	**105**	**106**	**109**
arable crop organic	17.2B24	103	**115**	**141**	102	98	**91**	101	**108**
arable crop organic	C2B2-1.C46	102	**116**	**112**	103	100	98	**112**	**107**
arable crop organic	FOS8500	100	100	100	100	100	100	100	100
cattle-based organic	17.461	104	**112**	**183**	**108**	**131**	**107**	98	**123**
cattle-based organic	17.2B24	96	98	**175**	103	**118**	99	**91**	**114**
cattle-based organic	C2B2-1.C46	**86**	**85**	**119**	**84**	**108**	**92**	**90**	**96**
cattle-based organic	FOS8500	100	100	100	100	100	100	100	100
cattle mono corn conv	17.461	98	**106**	**158**	**121**	**132**	**119**	**119**	**126**
cattle mono corn conv	17.2B24	**109**	**114**	**166**	**109**	**134**	104	**124**	**125**
cattle mono corn conv	C2B2-1.C46	**106**	**107**	**110**	**117**	**109**	**105**	**114**	**110**
cattle mono corn conv	FOS8500	100	100	100	100	100	100	100	100
average	17.461	100	**108**	**157**	**110**	**122**	**110**	**108**	**119**
average	17.2B24	103	**109**	**161**	**105**	**117**	98	**105**	**116**
average	C2B2-1.C46	98	103	**113**	102	**106**	98	**105**	**105**
average	FOS8500	100	100	100	100	100	100	100	100
average Mandaamin		100	**107**	**144**	**106**	**115**	102	**106**	**113**

The Mandaamin hybrids averaged 16% more uptake of microelements. On average they had 31% more Al, 4% less Cu, 19% more Fe, 5% more Mn, 19% more Si, 37% more Sr, 32% more Ti, and 8% less Zn than the FOS8500. The Mandaamin hybrids grown in the organic livestock system averaged 27% more uptake than the FOS8500 but the other two systems both averaged 11% more uptake.

Table 27. Total uptake of micromineras in hybrid corn grown in different farming systems in 2019.

		Al237	Cu	Fe	Mn	Si	Sr	Ti	Zn
arable crop organic	hybrid	lbs/acre							
arable crop organic	17.461	0.228	0.015	0.346	0.057	0.417	0.010	0.003	0.088
arable crop organic	17.2B24	0.293	0.012	0.544	0.053	0.548	0.011	0.007	0.074
arable crop organic	C2B2-1.C46	0.373	0.010	0.767	0.064	0.421	0.009	0.008	0.075
arable crop organic	FOS8500	0.223	0.013	0.467	0.060	0.445	0.009	0.004	0.099
cattle-based organic	17.461	0.454	0.032	0.775	0.085	0.732	0.011	0.007	0.124
cattle-based organic	17.2B24	0.521	0.024	0.700	0.115	0.665	0.013	0.009	0.097
cattle-based organic	C2B2-1.C46	0.452	0.023	0.595	0.073	0.547	0.009	0.007	0.105
cattle-based organic	FOS8500	0.326	0.021	0.525	0.079	0.512	0.007	0.007	0.101
cattle mono corn conv	17.461	0.445	0.032	0.543	0.072	0.762	0.018	0.006	0.190
cattle mono corn conv	17.2B24	0.414	0.026	0.609	0.073	0.715	0.019	0.006	0.113
cattle mono corn conv	C2B2-1.C46	0.518	0.029	0.641	0.059	0.577	0.014	0.007	0.127
cattle mono corn conv	FOS8500	0.401	0.046	0.555	0.067	0.541	0.012	0.004	0.161
average	17.461	0.38	0.03	0.55	0.07	0.64	0.01	0.01	0.13
average	17.2B24	0.41	0.02	0.62	0.08	0.64	0.01	0.01	0.09
average	C2B2-1.C46	0.45	0.02	0.67	0.07	0.52	0.01	0.01	0.10
average	FOS8500	0.32	0.03	0.52	0.07	0.50	0.01	0.01	0.12

Table 28. Total uptake of microelements relative to commercial check when grown in different systems in 2019.

		Al237	Cu	Fe	Mn	Si	Sr	Ti	Zn	average
system	hybrid	lbs/acre of micronutrients as % of FOS8500								
arable crop organic	17.461	102	120	**74**	**95**	94	121	78	89	97
arable crop organic	17.2B24	131	**90**	116	**88**	123	132	163	**74**	115
arable crop organic	C2B2-1.C46	167	**81**	164	108	**95**	102	188	**76**	123
arable crop organic	FOS8500	100	100	100	100	100	100	100	100	100
cattle-based organic	17.461	139	151	148	109	143	153	109	123	134
cattle-based organic	17.2B24	160	116	133	146	130	176	127	96	136
cattle-based organic	C2B2-1.C46	139	112	113	**93**	107	121	110	104	112
cattle-based organic	FOS8500	100	100	100	100	100	100	100	100	100
cattle mono corn conv	17.461	111	**71**	98	107	141	148	126	118	115
cattle mono corn conv	17.2B24	103	**57**	110	110	132	159	139	**70**	110
cattle mono corn conv	C2B2-1.C46	129	**64**	115	**88**	107	120	153	**78**	107
cattle mono corn conv	FOS8500	100	100	100	100	100	100	100	100	100
average	17.461	**118**	**114**	**106**	**104**	**126**	**141**	**104**	**110**	**115**
average	17.2B24	**131**	**88**	**120**	**115**	**128**	**156**	**143**	**80**	**120**
average	C2B2-1.C46	**145**	**86**	**131**	96	**103**	**114**	**150**	**86**	**114**
average	FOS8500	100	100	100	100	100	100	100	100	100
average Mandaamin		**131**	96	**119**	**105**	**119**	**137**	**132**	92	**116**

Analysis of variance showed significant differences between hybrids ($p < 0.0001$) and minerals ($p < 0.01$).

Table 29. Analysis of variance for total mineral uptake in the varietal trials of 2019 that evaluated the pedigree x mineral interaction.

Source	DF	Sum of Sq	F Ratio	Prob > F
system	2	1734	0.76	0.47
hybrid	3	30034	8.73	<.0001
system*hybrid	6	7375	1.07	0.38
mineral	14	34055	2.12	0.01
system*mineral	28	29297	0.91	0.60
hybrid*mineral	42	41798	0.87	0.70
system*hybrid*mineral	84	26225	0.27	1.00

The values presented in tab les x and y were based on ratios calculated from LS means. The data was reanalyzed by taking ratios between raw data then analyzing it. Based on least square means derived from that analysis of variance, on average the three hybrids 17.461, 17.2B24, and C2B2.C46 had 24%, 20%, and 12% greater mineral uptake relative to FOS8500. According to t tests the 17.461 did not differ significantly only from 17.2B24, which did not differ significantly from C2B2.C46. All of the Mandaamin hybrids differed significantly from FOS8500. These estimates are slightly higher than previous estimates probably due to not using LS means for calculating the initial ratios with the FOS8500 standard values.

A summary of the differences between hybrids and elements is shown in Table 30. the highest % increase was associated with the macroelements Ca and Mg and with microelements Al, Fe, Sr, Ti, and Si. The 17.461 and 17.2B24 hybrids had only 6 and 8% more C production than the FOS8500. However, these two hybrids respectively took up 62% and 63% more Ca, 36% and 25% more Al, 25 and 28% more Si, 45% and 55% more Sr, and 6% and 35% more Ti, than did the FOS8500. The high uptake of these minerals by the Mandaamin hybrids suggests enhanced mobilization of minerals from the solid portions of the soil including alumino-silicate clay particles, from parent mineral particles, and from limestone rather than from passive reliance on the soil solution.

Table 30. LS mean values for the relative uptake of nutrients by hybrids based on FOS8500 as a standard for the varietal trials in 2019.

	hybrid				
Element	FOS8500	17.461	17.2B24	C2B2-1.C46	Ave. Mandaamin
	uptake/acre in % relative to FOS8500				
Al	100	136	125	145	135
C	100	106	108	101	105
Ca	100	162	163	116	147
Cu	100	129	93	92	104
Fe	100	118	117	128	121
K	100	119	112	106	112
Mg	100	130	123	111	121
Mn	100	106	114	98	106
N	100	114	111	105	110
P	100	122	107	105	111
S	100	114	111	109	111
Si	100	125	128	102	118
Sr	100	145	155	117	139
Ti	100	106	135	149	130
Zn	100	130	94	100	108
Average	100	124	120	112	

Varietal trials in 2020. These trials tested nine different experimental hybrids ranging from 92 day to 108 day relative maturity. For the eight sites tested, we managed to obtain valid and complete data for five sites. Data analysis used an analysis of variance with hybrid as the main factor and plant population density and $\delta^{15}N$ as covariates. Utilization of covariates appeared necessary primarily because of deviations in plant population results and secondarily as a research tool to clarify the effects of $\delta^{15}N$ on variance. Results were that all three factors significantly affected grain production but only hybrid and $\delta^{15}N$ significantly affected protein, oil, starch, ethanol yield, lysine methionine, and cysteine. Population density did not affect grain quality. The $\delta^{15}N$ covariate had positive effects on grain yield, harvest index, grain protein, density, lysine, methionine, and cysteine, but negative effects on stover yield, grain oil, starch, and ethanol yield. Hence $\delta^{15}N$ was positively associated with protein accumulation and quality but negatively associated with non protein components. The portion of variance associated with $\delta^{15}N$ was significantly approximately half or more of the total sum of squares (SS) that were accounted by the model for harvest index, grain protein and amino acids, starch, ethanol yield, and density.

Yields and quality results are shown in Table 31 and 32. Grain yields and harvest index values were lower in 2020 than they had been in 2019 FOS8500, 17.2B24, and 17.461 averaged 177, 156, and 158 bu/acre in 2019 but they averaged 130, 96, and 128 bu/acre, respectively, in 2020. Harvest index for FOS8500, 17.2B24, and 17.461 averaged 64%, 58%, 57%, and 64% in 2019. But in 2020 they averaged 47%, 40%, and 37%, respectively. This implies that growing conditions in 2020 were stressful and this is corroborated by visual evidence of greater weed problems on the Goldstein (severe thistle infestation) and Beiler sites in 2020. For hybrids grown in 2020, yields were generally lower for the earlier hybrids with lower relative maturity (RM) ratings (see Table 31). An exception to this was the earliest hybrid

(K5N.NG10) which yielded 109 bu/acre. As in 2019, the 17.461 had the highest stover yield which was 40% higher than for the check. Though hybrid effects on harvest index were not statistically significant, the FOS8500 again had a higher value (15% more, p=0.153) than the other hybrids. The FOS8500 also had statistically significantly higher starch and ethanol yield than the Mandaamin hybrids but the magnitude of the difference was only +3%. The Mandaamin hybrids differed in their yields and quality characteristics between themselves and there were significant differences between them and the FOS8500. On average the FOS8500 had 17% less protein, 8% less oil, 15% less lysine, 23% less methionine, and 14% less cysteine than the average value for the Mandaamin hybrids.

Table 31. Yield and grain quality of nine hybrids grown on five sites in the varietal trials of 2020.

RM	Hybrid	Grain Yield				Stover Yield		Harvest Index		Grain Protein		Grain Oil		Grain Starch		Grain Density		Ethanol Yield		Lysine		Methionine		Cysteine	
days		bu/acre		lbs dm/acre				%								g/cm^3		gal/bu		% in whole grain					
105	FOS 8500	130	a	6,173	a	7,908	bcd	47.2	a	5.88	c	4.07	d	61.2	a	1.172	b	2.84	a	0.275	d	0.197	c	0.157	d
108	17.2B24	96	ab	4,558	ab	7,987	bc	39.8	a	6.65	bc	4.33	abcd	59.9	b	1.176	b	2.77	b	0.305	bc	0.239	ab	0.180	abc
105	17.461	128	a	6,033	a	11,077	a	37.0	a	6.72	bc	4.40	abc	59.9	b	1.191	ab	2.77	b	0.304	bc	0.239	ab	0.177	abc
105	UR65.461	93	ab	4,400	ab	8,218	bc	38.3	a	6.36	bc	4.58	a	59.8	bc	1.167	b	2.77	b	0.317	abc	0.228	b	0.169	cd
103	15.461	90	ab	4,241	ab	7,941	bcd	37.7	a	7.16	ab	4.15	cd	59.5	bc	1.209	a	2.74	bc	0.316	abc	0.259	a	0.187	a
97	9215.461	79	b	3,759	b	5,613	cd	45.0	a	7.19	ab	4.55	ab	59.0	cd	1.178	ab	2.73	bc	0.325	ab	0.246	ab	0.181	abc
96	924.461	83	b	3,928	b	7,410	bcd	38.7	a	6.29	bc	4.50	ab	59.9	b	1.179	ab	2.78	b	0.303	c	0.235	ab	0.171	bcd
96	924.NG10	73	b	3,464	b	5,144	d	44.0	a	6.94	ab	4.28	bcd	59.6	bc	1.173	b	2.75	bc	0.317	abc	0.242	ab	0.179	abc
92	K5N.NG10	112	ab	5,293	ab	9,505	ab	38.8	a	7.65	a	4.53	ab	58.6	d	1.168	b	2.70	c	0.336	a	0.254	a	0.186	ab
	ave. Mand	94		4,459		7,862		40		6.87		4.41		59.5		1.180		2.75		0.315		0.243		0.179	

Table 32. Yield and grain quality of nine hybrids grown on five sites relative to commercial check.

RM	Hybrid	Grain Yield		Stover Yield	Harvest Index	Grain Protein	Grain Oil	Grain Starch	Grain Density	Ethanol Yield	Lysine	Methionine	Cysteine
days		values as % of FOS8500											
105	FOS 8500	100	100	100	100	100	100	100	100	100	100	100	100
108	17.2B24	74	74	101	84	113	106	98	100	97	111	121	114
105	17.461	98	98	140	79	114	108	98	102	97	111	121	113
105	UR65.461	71	71	104	81	108	112	98	100	98	115	115	108
103	15.461	69	69	100	80	122	102	97	103	97	115	131	119
97	9215.461	61	61	71	95	122	112	96	101	96	118	125	115
96	924.461	64	64	94	82	107	110	98	101	98	110	119	109
96	924.NG10	56	56	65	93	118	105	97	100	97	115	123	114
92	K5N.NG10	86	86	120	82	130	111	96	100	95	122	129	118
	ave. Mand	72	72	99	85	117	108	97	101	97	115	123	114

Table 33. Analysis of variance for characteristics of hybrids grown in varietal trials in 2020.

		moist grain bu/acre		dry grain lbs/acre		stover lbs/acre		Harvest Index (%)		Grain Protein (%)		Grain Oil (%)	
Source	DF	SS	P	SS	P	SS	P	SS	P	SS	P	SS	P
Pedigree	8	14615	0.123	32725991	0.123	94790711	0.02	453.9095	0.838	11.6882	0.011	1.30352	0.0036
plant pop	1	25246	<.0001	56531432	<.0001	2.45E+08	<.0001	96.7532	0.356	0.50868	0.31	0.06052	0.2526
δ15NAir (‰)	1	6291	0.02	14086379	0.02	11972481	0.107	1093.459	0.004	46.0015	<.0001	0.69277	0.0004
% var hybrid		32		32		27		28		20		63	
% var pop		55		55		70		6		1		3	
% var δ^{15}N		14		14		3		67		79		34	
effect of δ^{15}N		positive		positive		negative		positive		positive		negative	
		Grain Starch (%)		Grain Density		Ethanol Yield		Lysine % whole		Methionine %		Cysteine %	
Source		SS		SS	P	SS	P	SS	P	SS	P	SS	P
Pedigree		21.0349	<.0001	0.006923	0.263	0.060538	0.003	0.011924	2E-04	0.01268	9E-04	0.00339	0.0064
plant pop		0.25997	0.436	2.6E-07	0.984	0.002891	0.24	0.000439	0.213	9E-05	0.616	6.6E-05	0.4777
δ15NAir (‰)		12.4965	<.0001	0.007492	0.002	0.123482	<.0001	0.011358	<.0001	0.01119	<.0001	0.01107	<.0001
% var hybrid		62		48		32		50		53		23	
% var pop		1		0		2		2		0		0	
% var δ^{15}N		37		52		66		48		47		76	
effect of δ^{15}N		negative		positive		negative		positive		positive		positive	

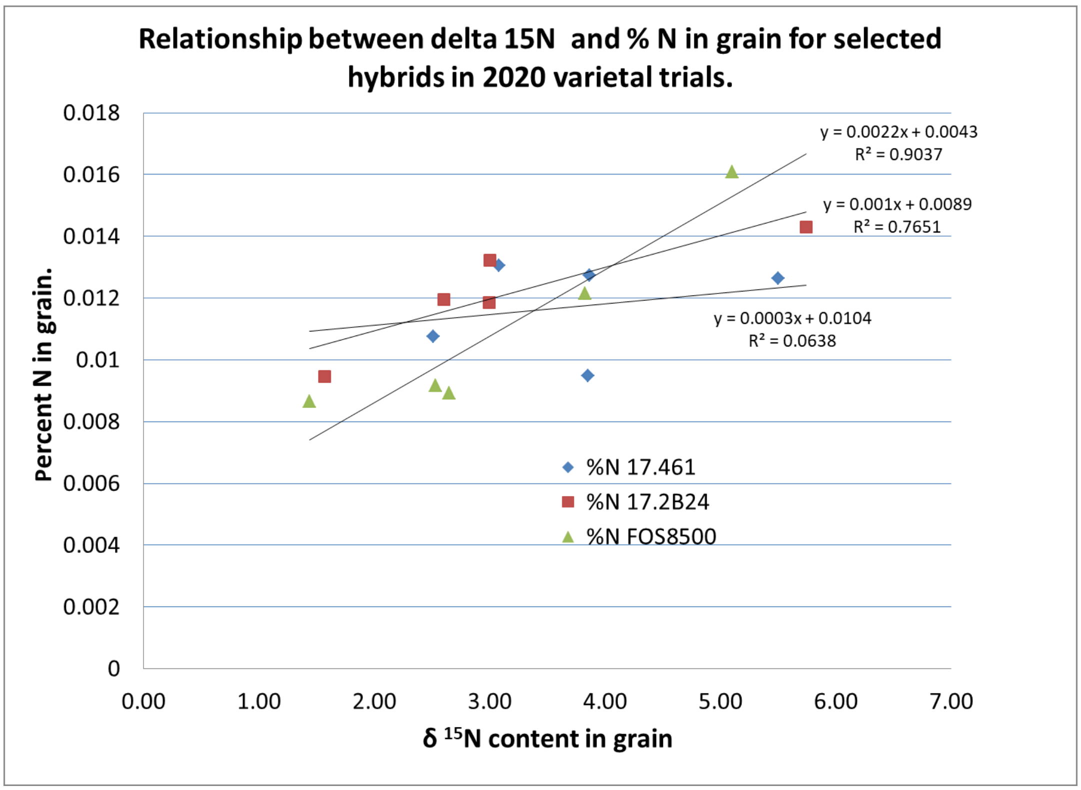
Variances associated with agronomic characteristics including covariant information on plant population density, δ^{15}N, %N content of grain, N uptake by grain, and plant population are shown in Table 32. There was no significant effect of hybrid pedigree if the anova model included only pedigree as a factor for testing δ^{15}N, %N content of grain, and pounds of N taken up by grain. If %N and %C in grain were taken as covariates in addition to pedigree the analysis of variance produced F values with p = 0.0001 for %N, p=0.0002 for %C, and p=0.0957 for pedigree. When δ^{15}N itself was analyzed with that same model, 80.2% of the total sum of squares was associated with %N. Conversely, Table 34 shows results obtained by analyzing %N in grain by pedigree, δ^{15}N, and plant population density. δ^{15}N accounted for large amounts of the total sum of squares. This again shows the tight relationship between δ^{15}N and %N or % protein in grain and the quixotic nature of these two parameters which describe source and magnitude of supply of nitrogen to the grain. They correlate with many parameters but are plastic factors that synthesize many processes and they have to be mutually considered when analyzing hybrid effects.

The relationship between δ^{15}N and the %N in the grain was tested for the individual hybrids using regression analysis despite the low sample sizes. 17.461 stood out in the trials for having similar yields to FOS8500 but the highest levels of δ^{15}N, %N in grain, and N uptake in grain (see least square means in Table 34). Regression coefficients showed a much higher intercept value and a lower slope coefficient than for the other hybrids. Though the slope coefficient was not significant nevertheless, the graphic representation of this data in a scatter-gram (Diagram 7) suggests that 17.461 has a higher and more stable content of N in grain that was maintained at higher δ^{15}N values.

Table 34. Agronomic characteristics and linear equations for hybrid trials in 2020.

	Agronomic characteristics.					Regression coefficients x=δ15N, y= %N in grain.						
Hybrid	no of plants/acre		δ15Nin grain	%N in grain	N uptake in grain lbs/a	intercept	std error	Prob>|t|	δ15N	std error	Prob>|t|	R^2
FOS8500	24,692	abc	3.11	1.15	73	0.0043	0.0014	0.0507	0.0022	0.0004	0.0131	0.9
17.2B24	24,427	bc	3.18	1.16	50	0.0089	0.0011	0.0043	0.0010	0.0003	0.0522	0.77
17.461	23,630	bc	**3.76**	**1.35**	**82**	**0.0104**	0.0030	0.0401	**0.0003**	0.0008	0.6819	0.06
UR56.461	25,091	abc	3.51	1.17	54	0.0036	0.0016	0.1007	0.0019	0.0004	0.0218	0.87
15.461	22,966	c	3.07	1.21	41	0.0070	0.0017	0.0279	0.0011	0.0005	0.1306	0.59
9215.461	30,268	a	3.36	1.31	72	0.0071	0.0018	0.027	0.0013	0.0005	0.0706	0.72
924.461	25,489	abc	3.52	1.25	55	0.0045	0.0009	0.0131	0.0021	0.0002	0.0027	0.97
924.NG10	29,073	ab	3.33	1.33	62	0.0078	0.0035	0.115	0.0013	0.0010	0.3127	0.33
K5N.NG10	20,577	c	3.40	1.40	60	0.0056	0.0028	0.1408	0.0022	0.0008	0.0741	0.71

Diagram 7. Relationships between δ^{15}N and %N for hybrids.



Stover production and composition in the 2020 variety trials:

Stover production and composition is shown in Table 35 and 36. The analysis of variance showed that though pedigree did not significantly affect δ^{15}N it affected stover yield, stover N, and %N. The δ^{15}N values significantly affected stover yield, stover N and %N as well. Though it accounted for little of the total variation in stover yield and stover N, it accounted for about half of the variation found in %N.

Table 35. Analysis of variance for stover parameters of the 2020 varietal trials, showing p values for significance and the % of the total SS associated with the different sources of variation.

		Parameter analyzed			
	DF	δ15N	Stover yield	Stover N	%N
Source variation		Prob > F			
Pedigree	8	0.6759	0.0547	0.104	0.026
δ15NAir (‰)	1		<.0001	<.0001	0.015
plants/a	1	0.0332	0.8064	0.2501	0.145
% of tot SS from Pedigree		54	28	30	21
% of tot SS from δ15N			0.1	3	53

The hybrids that produced the most stover were 17.461 and K5N.NG10. These two hybrids appeared to pursue a very different investment strategy than did the FOS8500 in terms of growth allocation. They had very high δ^{15}N contents in their stalks, produced a lot of stover with relatively large amounts of N (see Table 36) as well as Ca in the stover. They possessed average %N in the stover but produced grain with high protein contents. The plants seemed to be investing in rhizophagy, vegetative growth, and loading N, Ca, and other minerals into grain. The 17.461 had 53% higher δ^{15}N, 36% higher stover yield, 37% higher stover N, but the same %N in its stover as did the FOS8500. The K5N.NG10 had 72% higher δ^{15}N, 17% higher stover yield, 58% higher stover N, and 45% higher %N in its stover as did the FOS8500 Similar data with respect to stover production were obtained in 2019 with the Mandaamin hybrids, especially with 17.461.

Table 36. Effects of hybrids on stover yield and N content; varietal trials 2020.

hybrid	δ15N		Stover yield		Stover N		N	
	(‰)		lbs/acre				%	
FOS8500	1.66	a	7993	bc	59	abc	0.677	b
17.2B24	1.71	a	8029	abc	49	bc	0.601	b
17.461	2.53	a	10863	a	81	ab	0.677	b
UR65.641	1.81	a	8131	abc	58	abc	0.699	b
15.461	1.86	a	7994	bc	56	bc	0.675	b
9215.461	1.28	a	5758	c	32	c	0.596	b
924.461	2.40	a	7374	bc	59	abc	0.705	b
924.NG10	1.61	a	5282	c	30	c	0.646	b
K5N.NG10	2.86	a	9378	ab	93	a	0.979	a
data as % of FOS8500 control								
FOS8500	100		100		100		100	
17.2B24	103		100		83		89	
17.461	153		136		137		100	
UR65.641	109		102		98		103	
15.461	112		100		95		100	
9215.461	77		72		54		88	
924.461	144		92		101		104	
924.NG10	97		66		51		96	
K5N.NG10	172		117		158		145	

Nutrient composition of grain in 2020 variety trials: The nutrient concentrations of grain and stalks were also analyzed using the factor hybrid pedigree and the covariates $\delta^{15}N$ and plant population density. Values showed substantial variation was accounted for by all three factors. Pedigree accounted for an average of 54% of the total sum of squares while $\delta^{15}N$ accounted for an average of 34% of the total sum of squares.

Table 37. Results of an analysis of variance of grain composition analyzing pedigree, plants/acre, $\delta^{15}N$, and plant density for varietal trials in 2020.

		%N	%C	Ca	Cu	Fe	K	Mg	Mn	P177	S180	Zn
Source	DF	Prob > F										
Pedigree	8	**0.0699**	**0.0059**	0.195	0.143	0.2992	0.2569	0.212	0.1128	**0.0026**	0.179	0.337
δ15NAir (‰)	1	**<.0001**	0.1546	0.517	**0.095**	**0.0001**	**0.0736**	**<.0001**	0.3989	**<.0001**	**0.002**	**0.01**
plants/a	1	0.2379	0.948	**0.01**	**0.016**	**0.0214**	0.942	**0.03**	0.1115	0.3735	0.346	0.839
% of tot SS from Pedigree		18	93	60	59	29	76	31	81	38	50	56
% of tot SS from δ15N		81	7	2	13	54	24	55	4	61	46	44
relation with δ15N		positive			positive	positive	positive	positive		positive	positive	positive

The concentration of nutrients in grain for different hybrids are shown in Table 38 . Their value in percent, relative to FOS8500 are shown in Table 39. The Mandaamin hybrids averaged 27% more minerals in their grain. This consisted mainly in 63% more Ca, 20% more Cu, 22% more Mn, 10% more P, 14% more S, 24% more Zn, and 9% more N. However, the Mandaamin hybrids had 25% less Fe and 5% less K than the check. As had been the case in 2019, 17.461 led the other hybrids in terms of concentration of nutrients in its grain. On average it averaged 61% higher nutrient values in its grain than the check. This included 270% more Ca, 99% more Cu, 10% more Mg, 41% more Mn, 9% more P, 19% more S, and 36% more Zn. High relative levels were distributed amongst different kinds of minerals. As yields of 17.461 were very similar to FOS8500 these very large differences are clearly not due to yield reduction and some kind of nutrient concentration effect.

Table 38. Quantities of mineral components in grain of different hybrids in varietal trials 2020.

Hybrid	δ15N	N	C	Ca	Cu	Fe	K	Mg	Mn	P177	S180	Zn
		%		concentration in grain in µg/g								
FOS 8500	3.11	1.19	45.41	29.9	1.10	29.4	4347	1142	4.35	3079	969	18.7
17.2B24	3.18	1.19	45.63	38.8	0.93	22.1	4337	1156	4.91	3233	1033	22.1
17.461	**3.76**	1.30	45.61	110.6	2.18	23.3	4120	1254	6.12	3356	1155	25.4
UR65.461	3.51	1.15	45.70	40.8	1.13	19.4	3978	1121	5.20	3193	1081	19.2
15.461	3.07	1.27	45.59	45.2	1.74	24.7	4236	1135	5.05	3334	1107	26.6
9215.461	3.36	1.28	45.72	25.3	0.96	20.4	4279	1262	4.64	3777	1183	23.9
924.461	3.52	1.22	45.63	53.3	1.16	23.7	4132	1242	5.47	3398	1080	25.1
924.NG10	3.33	1.31	45.68	15.5	0.92	19.7	4010	1233	5.04	3367	1137	21.1
K5N.NG10	3.40	1.43	45.90	61.1	1.52	23.9	3951	1218	5.90	3368	1074	23.0

Table 39. Relationships between Mandaamin hybrids and a hybrid check for mineral composition of grain; varietal trials 2020.

	δ15N	%N	%C	Ca	Cu	Fe	K	Mg	Mn	P177	S180	Zn	ave. minerals
Level	values in % of relevant FOS8500 check value.												
FOS 8500	100	100	100	100	100	100	100	100	100	100	100	100	**100**
17.2B24	102	100	100	130	**84**	**75**	100	101	113	105	107	118	**105**
17.461	121	109	100	**370**	**199**	**79**	**95**	**110**	**141**	**109**	**119**	**136**	**161**
UR65.461	113	**97**	101	136	103	**66**	**92**	**98**	119	104	111	103	**105**
15.461	**99**	106	100	151	158	**84**	**97**	**99**	116	108	114	142	**119**
9215.461	108	108	101	**84**	**87**	**69**	**98**	111	107	123	122	128	**102**
924.461	113	103	100	178	105	**81**	**95**	109	126	110	111	134	**119**
924.NG10	107	110	101	**52**	**83**	**67**	**92**	108	116	109	117	113	**93**
K5N.NG10	109	120	101	204	138	**81**	**91**	107	136	109	111	123	**127**
ave. Mandaamin	109	107	101	163	120	**75**	**95**	105	122	110	114	124	117

These values in the table above represent ratios of least square means generated by the anova model with covariates. The ratios based on raw values relative to FOS8500 were re-analyzed for significance using an analysis of variance with hybrids, minerals and hybrids x minerals as factors and plant population and $\delta^{15}N$ values as covariates. Additional ICP data were included for P and S at two different wavelengths. Contrasts were used to compare the different kinds of hybrids. The analysis of variance in Table 40 shows that approximately half of the sum of squares (SS) variance was attributed to the pedigree x mineral interaction, and most of the rest to pedigree and mineral.

Table 40. Analysis of variance of relative values for mineral composition of grain with FOS8500 as the standard for varietal trials in 2020.

Source	DF	SS	F Ratio	Prob > F
Pedigree	8	159194	5.4968	<.0001
plant pop	1	14299	3.9498	0.0474
δ15NAir (‰)	1	23844	6.5865	0.0106
mineral	13	190914	4.0567	<.0001
Pedigree*mineral	104	359411	0.9546	0.6062
%ss for pedigree		21		
%ss for delta 15N		3		
% ss for mineral		26		
% ss for pedigree x mineral		48		

The table 41 showing the interaction between nutrient and pedigree shows the same major kinds of differences as were shown in Table 39 , with minor differences.

Table 41 shows relative values for nutrient composition of grain based on an analysis of variance with pedigree, mineral, pedigree x mineral as main factors and $\delta^{15}N$, and plant population density as covariates.

	Hybrid									
Mineral	FOS8500	17.2B24	17.461	UR65.461	15.461	9215.461	924.461	924.NG10	K5N.NG10	
	% relative to FOS8500 based on means									ave Mand
%C	99	100	105	102	101	94	101	95	107	101
%N	99	105	125	105	109	110	110	114	131	113
Ba	99	59	**282**	103	66	88	97	117	103	114
Ca	99	125	**355**	139	124	172	190	119	137	170
Cu	99	83	**223**	110	154	125	118	114	131	132
Fe	99	85	99	82	90	82	96	86	96	90
K	99	100	101	93	99	93	97	88	98	96
Mg	99	102	119	101	102	101	111	102	118	107
Mn	99	114	147	123	116	110	129	120	140	125
P 177	99	106	119	108	111	117	114	106	119	112
P 213	99	105	118	107	110	116	114	105	118	112
S 180	99	107	128	114	116	115	114	111	120	116
S 181	99	103	123	109	112	113	114	108	116	112
Zn	99	128	164	108	163	137	162	130	142	142
std error	27	27	27	27	27	27	27	27	27	
average	99	102	158	107	113	112	119	108	120	117
average min -C	99	102	162	108	113	114	120	109	121	119
ave.-C, P213, S181	**99**	**101**	**169**	**108**	**114**	**114**	**122**	**110**	**121**	120

The hybrids with 461 as a parent averaged 122% while the FOS8500 averaged 99%. The contrast was significantly different at p=0.0043. The hybrids with NG10 as a parent averaged 114%. The contrast with FOS8500 was p=0.0636. The 17.461 averaged 158% while the 172B24 averaged 102 and the difference was significant at p = 0.0001. Hybrids with 461 as a parent did not differ significantly from hybrids with NG10 as a parent (p = 0.195).

Nutrient composition of stalks in 2020 variety trials: The analysis of variance for nutrient composition of stover is shown in Table 42 based on Pedigree as main factor and δ^{15}N and plants/acre as covariates. The mean values and relative values are shown in Tables 43 and 44. On average 52% of the total SS associated with variation for the various components was associated with pedigree and 26% was associated with δ^{15}N. However, statistically significant effects of pedigree were found only for Mg while significant effects of δ^{15}N and plants/acre were found for most of the parameters tested.

Table 42. Analysis of variance table for stover composition results from 2020 variety trials.

		%N	%C	Ca	Cu	Fe	K	Mg	Mn	P177	S180	Zn
Source	DF	Prob > F										
Pedigree	8	0.1447	0.2744	0.3481	0.7573	0.6639	0.7175	**0.0556**	0.7191	0.3411	0.9059	0.8267
δ15NAir (‰)	1	**0.0147**	**0.088**	0.8617	**0.0246**	0.5996	**0.0072**	**<.0001**	**0.1036**	**0.0257**	0.3492	**0.0639**
plants/a	1	**0.0264**	**0.0237**	**0.0041**	**0.0055**	0.7542	**0.0005**	**0.0967**	**0.0582**	0.4851	0.9111	0.7334
% of tot SS from Pedigree		53	55	49	26	94	19	36	44	61	78	53
% of tot SS from δ^{15}N		26	16	0.2	28.7	4.5	28.9	58.1	23.4	35.4	21.4	45.8
rel with δ^{15}N		positive	positive		positive		positive	negativ	negativ	negative		negative

Table 43. LS mean values for stover composition results from 2020 variety trials.

Hybrid	%N	%C	Ca	Cu	Fe	K	Mg	Mn	P177	S180	Zn
	concentration of minerals in µg/g										
FOS 8500	0.677	45.04	2176	4.88	259	11509	1871	42.5	2928	635	53.9
17.2B24	0.601	45.03	2721	3.72	229	11685	1954	41.3	2764	637	49.2
17.461	0.677	44.47	2551	4.80	228	13715	2099	37.1	3594	729	57.8
UR65.461	0.699	44.65	2599	4.59	248	11351	2346	44.6	3848	807	64.5
15.461	0.675	45.15	2349	4.87	163	11749	1880	39.8	3217	659	46.8
9215.461	0.596	45.20	2127	3.57	204	10128	2352	29.2	3103	752	48.5
924.461	0.705	44.70	2752	3.52	213	10333	2402	37.6	3310	757	49.0
924.NG10	0.646	44.66	2365	3.81	172	10797	2281	39.8	3709	821	59.2
K5N.NG10	0.979	44.69	2315	5.03	195	13771	2031	46.1	3703	845	52.4

Table 44. LS mean values for stover composition results from 2020 variety trials relative to the check.

Hybrid	%N	%C	Ca	Cu	Fe	K	Mg	Mn	P177	S180	Zn	ave min.
	values in % of relevant FOS8500 check value.											
FOS 8500	100	100	100	100	100	100	100	100	100	100	100	100
17.2B24	89	100	125	76	89	102	104	97	94	100	91	97
17.461	100	99	117	98	88	119	112	87	123	115	107	107
UR56.461	103	99	119	94	96	99	125	105	131	127	120	112
15.461	100	100	108	100	63	102	100	93	110	104	87	97
9215.461	88	100	98	73	79	88	126	69	106	118	90	93
924.461	104	99	126	72	82	90	128	88	113	119	91	101
924.NG10	96	99	109	78	67	94	122	94	127	129	110	102
K5N.NG10	145	99	106	103	75	120	109	108	126	133	97	112
average Mand.	103	100	114	87	80	102	116	93	116	118	99	103

The results from stover mineral analysis suggested that there was much less difference between the Mandaamin hybrids and the check for stover than had been present for grain. On average the Mandaamin hybrids had only 3% more minerals than did the check. The major differences were again for that the Mandaamin hybrids There were 14-18% increases for Ca, Mg, P, and S but 13 and 20% less Cu and Fe.

A fuller analysis of variance took into account the pedigree x mineral interaction, more minerals, and the usual covariates (see Table 45). The analysis showed significant effects for pedigree, minerals, δ^{15}N, and plants/acre. Most variation was found for mineral and mineral x pedigree.

Table 45. Analysis of variance considering minerals as a factor. Hybrid trials 2020.

Source	DF	SS	F Ratio	Prob > F
Pedigree	8	16279.08	2.7484	**0.0056**
mineral	13	46246.32	4.8048	**<.0001**
Pedigree*mineral	104	43896.73	0.5701	0.9997
δ15NAir (‰)	1	6911.476	9.335	**0.0024**
plants/a	1	3836.774	5.1822	**0.0232**

The effects of pedigree as shown in Table 46 indicates that UR65.461, another 461 hybrid with 105 day RM, had the highest concentrations of minerals in its stover. However, this did not seem to be conveyed to its grain (see Table 38). On the other hand, the two Mandaamin hybrids with the highest stover and grain yields (17.461 and K5N.NG10) had also high relative concentrations of minerals in their stover and grain. There was a positive correlation between relative concentration of minerals in grain and the stover yield (R^2 = 0.48; p = 0.056) as well as for the grain yield (R^2 = 0.56; p = 0.032).

Table 46 shows least square values for the concentration of minerals in stover and grain relative to FOS8500 and the actual stover and grain yields.

Hybrid	Stover conc.		Grain conc.		Stover yield		Grain yield	
	% relative to FOS8500				lbs dm/acre			
FOS 8500	101	c	99	b	7,908	bcd	6,173	a
17.2B24	103	bc	102	b	7,987	bc	4,558	ab
17.461	107	bc	158	a	11,077	a	6,033	a
UR56.461	118	a	107	b	8,218	bc	4,400	ab
15.461	103	bc	113	b	7,941	bcd	4,241	ab
9215.461	102	bc	112	b	5,613	cd	3,759	b
924.461	103	bc	119	b	7,410	bcd	3,928	b
924.NG10	109	abc	108	b	5,144	d	3,464	b
K5N.NG10	111	ab	120	b	9,505	ab	5,293	ab

The full interaction between hybrid pedigree and mineral type is shown in Table 47. The pattern shown is similar to that shown in Table 46. The differences between Mandaamin hybrids and FOS8500 ranged from 1 to 16%. On average the Mandaamin hybrids had 6% more minerals than the FOS8500 in their stover whereas the grain of the same hybrids averaged 17% more minerals than the FOS8500 in their grain.

Table 47. Total mineral uptake by stover for different hybrids as a % of the FOS8500 check. 2020 variety trials.

	Hybrid									
Mineral	FOS8500	17.2B24	17.461	UR65.461	15.461	9215.5	924.461	924.NG10	K5N.NG10	
	% relative to FOS8500 based on LS means									ave Mand
%C	101	102	99	100	103	98	97	96	102	99
%N	101	96	109	113	106	116	106	116	149	114
Ba	101	112	91	102	91	101	90	98	92	97
Ca	101	126	115	121	106	128	109	117	98	115
Cu	101	79	104	104	100	84	98	97	96	95
Fe	101	116	99	125	92	93	105	88	95	102
K	101	103	125	104	96	100	105	110	109	106
Mg	101	109	103	126	108	115	114	114	108	112
Mn	101	98	82	103	93	82	78	100	96	91
P 177	101	97	115	130	115	104	97	119	125	113
P 213	101	97	116	132	114	104	98	118	125	113
S 180	101	106	113	134	113	120	122	124	130	120
S 181	101	108	118	138	115	119	122	127	136	123
Zn	101	94	106	119	90	83	88	101	95	97
std error	12	12	12	12	12	12	12	12	12	
average	101	103	107	118	103	103	102	109	111	107
average min -C	101	103	107	119	103	104	102	110	112	108
ave.-C, P213, S181	**101**	**103**	**106**	**116**	**101**	**102**	**101**	**108**	**108**	106

Total uptake of minerals in 2020 varietal trials.

An analysis of variance took into account all the minerals in the analysis. The models utilized hybrid pedigree, mineral, pedigree x mineral, with $\delta^{15}N$ in grain, $\delta^{15}N$ in stover, and plants/acre as covariates. The analysis of variance for total uptake of nutrient (Table 48) showed significant effects of pedigree, mineral, and of all the covariates (plants/acre, and $\delta^{15}N$ in stover and grain. Pedigree accounted for 49% of the total SS.

Table 48. Analysis of variance for total mineral uptake in lbs/acre. Varietal trials, 2020.

Source	Nparm	DF	SS	F Ratio	Prob > F
Pedigree	8	8	115386	15.6	**<.0001**
mineral	13	13	20145	1.7	**0.0617**
Pedigree x mineral	104	104	26329	0.3	1.0000
plants/a	1	1	40691	44.1	**<.0001**
δ15N stalk	1	1	23579	25.6	**<.0001**
δ15N grain	1	1	10556	11.4	**0.0008**
%ss pedigree			49		
% ss ped x min			11		
%ss δ15N			9		

The effect of hybrids is shown in the form of least square means in Table 49. The later maturing hybrids tended to have higher nutrient uptake values. 17.461 had 132% which was 29% higher than for the check FOS8500 which had 103%. LS means for the hybrid pedigree x mineral interaction are shown in Table 50.

Table 49. Relative uptake of minerals for different hybrids using mineral as a factor In analysis.

Hybrid	%	
FOS 8500	103	c
17.2B24	101	cd
17.461	132	a
UR65.461	119	b
15.461	89	e
9215.461	101	cd
924.461	87	e
924.NG10	94	cde
K5N.NG10	91	de

Table 50. LS mean values for the hybrid x mineral interaction for uptake of minerals in grain and stover and grain + stover for varieties grown in 2020.

	Ba	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**P 177**	**P 213**	**S 180**	**S 182**	**Zn**	**N**	**C**
Hybrid	uptake by grain in lbs/acre													
FOS 8500	0.0037	0.167	0.00629	0.182	25.6	6.7	0.026	18.3	18.5	5.75	6.20	0.122	72.9	2685
17.2B24	0.00075	0.145	0.00363	0.086	17.8	4.82	0.020	13.5	13.5	4.43	4.46	0.087	50.1	1940
17.461	0.01348	0.845	0.01611	0.151	24.2	7.56	0.038	20.1	20.1	7.04	7.21	0.158	81.7	2645
UR65.461	0.00166	0.172	0.00548	0.090	17.6	5.12	0.023	14.7	14.7	5.03	5.02	0.084	54.1	2041
15.461	0.00072	0.120	0.00532	0.077	14.4	3.84	0.016	11.3	11.3	3.82	3.88	0.087	41.5	1566
9215.461	0.00196	0.278	0.00773	0.141	22.7	6.38	0.027	19.9	19.9	6.10	6.45	0.131	72.1	2422
924.461	0.00219	0.253	0.00588	0.110	17.0	4.99	0.023	14.1	14.2	4.50	4.83	0.099	54.5	1884
924.NG10	0.00128	0.163	0.00554	0.102	18.7	5.46	0.024	15.5	15.6	5.17	5.33	0.095	62.5	2112
K5N.NG10	0.00107	0.146	0.00508	0.091	15.8	5.04	0.022	13.9	14.0	4.48	4.62	0.095	59.5	1815
average Mand.	**0.00289**	**0.265**	**0.00685**	**0.106**	**18.5**	**5.40**	**0.024**	**15.4**	**15.4**	**5.07**	**5.22**	**0.105**	**59.5**	**2053**

	Ba	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**P 177**	**P 213**	**S 180**	**S 182**	**Zn**	**N**	**C**
Hybrid	uptake by stover in lbs/acre													
FOS 8500	0.1385	17.08	0.03780	1.895	88.5	14.63	0.347	22.4	22.3	5.01	4.92	0.415	89.8	3524
17.2B24	0.1615	20.71	0.02496	1.782	83.5	15.46	0.337	21.3	21.1	4.83	4.92	0.374	87.0	3482
17.461	0.1714	25.19	0.05323	2.084	141.9	19.01	0.329	32.2	32.2	7.12	7.39	0.529	139.1	4536
UR65.461	0.1424	21.04	0.03570	2.042	87.5	19.35	0.364	31.6	31.8	6.62	6.93	0.533	94.6	3709
15.461	0.1075	14.65	0.02664	1.081	69.8	13.49	0.262	22.9	22.6	4.29	4.43	0.318	79.9	3069
9215.461	0.1649	23.44	0.04415	2.098	129.2	18.85	0.337	24.7	24.7	6.38	6.52	0.389	119.2	4030
924.461	0.1469	21.71	0.03433	1.603	95.7	16.31	0.303	23.4	23.4	5.38	5.50	0.330	96.0	3447
924.NG10	0.1555	21.59	0.03855	1.468	109.1	16.59	0.364	27.2	26.9	5.99	6.12	0.438	101.9	3476
K5N.NG10	0.1140	14.70	0.03371	1.300	87.8	13.32	0.285	24.2	24.0	5.70	5.94	0.349	97.8	3054
average Mand.	**0.14551**	**20.379**	**0.03641**	**1.682**	**100.6**	**16.55**	**0.323**	**25.9**	**25.8**	**5.79**	**5.97**	**0.408**	**102.0**	**3600**

	Ba	Ca	Cu	Fe	K	Mg	Mn	P 177	P 213	S 180	S 182	Zn	N	C
Hybrid	uptake by grain and stover in lbs/acre													
FOS 8500	0.1422	17.25	0.04	2.08	114	21.3	0.372	40.7	40.7	10.76	11.1	0.536	163	6209
17.2B24	0.1623	20.86	0.0286	1.869	101	20.28	0.357	34.8	34.7	9.26	9.4	0.461	137.1	5422
17.461	0.1848	26.03	0.0693	2.235	166	26.58	0.367	52.4	52.3	14.16	14.6	0.686	220.8	7181
UR65.461	0.1440	21.22	0.0412	2.132	105	24.48	0.387	46.2	46.5	11.65	12.0	0.617	148.7	5750
15.461	0.1082	14.77	0.0320	1.158	84	17.33	0.279	34.1	34.0	8.11	8.3	0.405	121.4	4635
9215.461	0.1669	23.71	0.0519	2.240	152	25.23	0.363	44.6	44.6	12.48	13.0	0.520	191.3	6451
924.461	0.1491	21.97	0.0402	1.713	113	21.30	0.326	37.5	37.6	9.87	10.3	0.430	150.5	5331
924.NG10	0.1568	21.76	0.0441	1.570	128	22.06	0.389	42.8	42.6	11.16	11.4	0.533	164.4	5588
K5N.NG10	0.1151	14.85	0.0388	1.392	104	18.36	0.307	38.1	38.0	10.18	10.6	0.444	157.4	4869
average Mand.	**0.14840**	**20.645**	**0.04325**	**1.788**	**119.1**	**21.95**	**0.347**	**41.3**	**41.3**	**10.86**	**11.19**	**0.512**	**161.5**	**5653**

The data for the interaction between hybrids and minerals is shown in Table 49 relative to the FOS8500 check. On average the Mandaamin hybrids showed especially higher uptake of Calcium.

Table 51. Total uptake of minerals by hybrids in 2020 as % of FOS8500.

	FOS8500	17.2B24	17.461	UR56.461	15.461	9215.461	924.461	924.NG10	K5N.NG10	Ave. Mand.
Mineral	% relative to FOS8500 based on LS means									
Ba	103	114	123	109	82	92	91	89	84	**98**
C	103	101	120	107	87	92	83	83	84	**95**
Ca	103	131	157	135	101	117	117	115	92	**121**
Cu	103	82	143	119	97	100	76	89	86	**99**
Fe	103	103	128	113	69	112	79	85	86	**97**
K	103	102	150	110	87	108	84	97	91	**103**
Mg	103	101	125	121	90	106	93	94	90	**103**
Mn	103	98	112	110	84	83	75	98	89	**94**
N	103	105	126	114	84	98	92	97	110	**103**
P 177	103	94	131	125	95	99	87	94	95	**102**
P 213	103	94	131	126	95	100	87	93	95	**102**
S 180	103	98	132	125	92	109	88	96	97	**105**
S 182	103	99	132	123	92	110	88	97	97	**105**
Zn	103	98	134	127	87	88	77	90	84	**98**
Std error	14	14	14	14	14	14	14	14	14	**14**
ave.	**103**	**101**	**132**	**119**	**89**	**101**	**87**	**94**	**91**	**102**
ave. -C	103	101	133	120	89	102	87	95	92	**102**
ave.-C,P213,S182	103	102	133	119	88	101	87	95	91	**102**

Harvest index for minerals; varietal trials 2020.

The higher yielding Mandaamin hybrids appeared to have allocated more nutrients into grain than the check. To test whether this had to do with partitioning of the mineral from stover to grain we calculated the harvest index values for the minerals taken up by the corn. We analyzed harvest index for each mineral. In the anova the main factor was hybrid pedigree and the covariates were plants/acre and the $\delta^{15}N$ in stover and grain. Table 52 shows that though pedigree accounted for 26% (range 16 to 75%) of the total variation, it was not a statistically significant effect. Plants/acre accounted for an average of

6% of the variation and the effect was not significant for any minerals. However the $\delta^{15}N$ in stover and grain accounted on average for 58% of the total SS variation and in very many cases, especially for the $\delta^{15}N$ in grain, the effects were statistically significant. Thus on the individual mineral basis, $\delta^{15}N$ was playing the predominant role in determining harvest index. The effect of $\delta^{15}N$ in grain on overall harvest index was positive for most minerals. The effect of $\delta^{15}N$ in stover on overall harvest index was negative for most minerals. Though effects of both kinds of $\delta^{15}N$ were not statistically significant for calcium and effects of $\delta^{15}N$ in stover were not statistically significant for Cu and Fe, these minerals still had the same pattern of positive responses of HI to $\delta^{15}N$ in grain and negative responses to $\delta^{15}N$ in stover. This may be due to a source-sink relationships where stover supplies grain with $\delta^{15}N$ and minerals.

Table 52. Probabilities and percent allocation of sums of squares for analysis of variance of harvest indices with pedigree as a main factor and covariates.

Anova Factor	Ba	Ca	Cu	Fe	K	Mg	Mn	P 177	P 213	S 180	Zn	%N	%C	Average
	Prob > F													
Pedigree	0.374	0.6678	0.7268	0.6629	0.7006	0.3621	0.8259	0.4198	0.3884	0.4208	0.3337	0.808	0.808	**0.5768**
δ15N grain	0.5358	0.4387	**0.0174**	**0.0258**	**0.0274**	**<.0001**	**0.0049**	**0.0003**	**0.0003**	**<.0001**	**0.0125**	**0.0004**	**0.0004**	**0.0967**
δ15N stalk	0.8673	0.5324	0.1289	0.1687	**0.0368**	**0.0689**	**0.0652**	**0.0876**	**0.0856**	**0.0214**	0.9051	**0.0097**	**0.0097**	**0.2298**
plants/a	0.1155	0.2157	0.2384	0.9156	0.0852	0.8699	0.4304	0.9854	0.9984	0.2931	0.5352	0.7804	0.7804	**0.5572**
SS% Pedigree	75	69	34	44	29	26	24	30	31	22	56	16	16	**36**
SS% δ15N	3	12	57	56	54	74	72	70	69	75	41	84	84	**58**
SS% Plants/a	22	19	9	0	17	0	4	0	0	3	2	0	0	**6**
rel with δ15N grain			positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	
rel. with δ15N stalk					negative	negative	negative	negative	negative	negative	negative	negative	negative	

Results Manure/Inoculation trials, 2018: In 2018 two hybrids were compared on three farms. These farms are coded as Beiler, Clark, and Anon, and they are located near the towns of Rewey, Spring Green, and Fayette, WI. Towards the end of the season the experimental hybrid C461.C2B2 showed it was susceptible to an infestation of the fungal disease Grey Leaf Spot (caused by *Cercospora zeae-maydis*) which was present in pandemic proportions on all sites. This disease decimated foliage on all sites during grain fill and undoubtedly contributed to premature dry down and lower yields.

Analysis of variance for grain yield showed significant treatment and interaction effects only for farm, hybrids, and farm x hybrid interactions (all at $p < 0.0001$). The population density covariate was significant at $p < 0.0096$. There was no significant effect of manure or of inoculation on the sites.

Yield results for the significant farm x hybrid interaction are shown in Table 53. On average, the FOS8507 yielded 123.2 bu/acre. On average, the C46.C2B4 yielded 92.4 bu/acre. Undoubtedly a portion of this decrease was due to disease susceptibility as previous trials had shown competitive yields under more normal yield conditions. These results caused us to switch Mandaamin hybrids in subsequent years.

Table 53. Results of manure and inoculation trials on three farms in 2018.

Grain yield for farms and hybrids on 3 farms in 2018.

Farm	Hybrid	group	bu/acre

S	FOS8507	a	174.4
S	C461.C2B4	b	124.3
B	FOS8507	b	135.7
B	C461.C2B4	c	95.1
C	FOS8507	d	59.6
C	C461.C2B4	d	57.6

Values not associated with the same group are statistically significantly different at the p<0.05 level.

Anibas Farm inoculation study 2019. The same design was applied in 2019 on the Anibas farm. However, all plots were manured with dairy manure and there were only the hybrid and inoculation treatments. Grain was harvested as for 2018. However, roots and stalks were harvested from the same areas as yield shredded ground, and submitted to USDA in Morris MN for mineral analysis with ICP and C and N analysis with a Leco analyzer. Results from the analysis of variance for grain yield are shown in Table 54.

Analysis of yield results showed that inoculation had no effect on yield but hybrids differed significantly. The FOS8500 yielded 152.3 bu/acre and the 17.461 yielded 125.7 bu/acre. Results from the mineral analysis are shown in Table 55. There was no direct effect of inoculation on mineral nutrient accumulation. The main significant factors in analyses of variance, when they occurred, were or the hybrid, the part of the plant sampled, and the interaction between the hybrid and the part of the plant. These present for some elements and not for other (see Table 54).

Table 54. P level of significance or the effect of factors hybrid, parts (grain, stalk, roots) and the hybrid x part interaction for farm A in 2019.

Nutrient	hybrid	part	part x hybrid
%N	0.9154	<.0001	0.0005
%C	<.0001	<.0001	<.0001
Ca	0.2898	<.0001	0.6168
Al 237.312	0.0871	<.0001	0.0995
Al 394.401	0.0902	<.0001	0.0876
Copper	0.219	0.0019	0.0655
Iron	0.6126	<.0001	0.7943
Potassium	<.0001	<.0001	<.0001
Magnesium	<.0001	<.0001	<.0001
Manganese	0.8388	<.0001	0.8011
P177.434	0.27	<.0001	0.0027
P213.62	0.309	<.0001	0.0026
S 180.669	<.0001	<.0001	<.0001
S 181.972	<.0001	<.0001	<.0001
Silicon	0.0667	0.0668	0.0319
Zinc	0.0339	0.0097	0.0369

The results indicate profound differences in uptake and mobilization of certain nutrients, and their accumulation in grain. The hybrid 17.461 mobilized higher concentrations of nitrogen, carbon, phosphorus, and sulfur into its grain than did FOS8500 (see Table x). FOS 8500 tended to accumulate higher levels of potassium, magnesium, and sulfur in its roots than 17.461. Conversely, 17.461 accumulated higher levels of carbon, aluminum, copper and zinc in its roots and silica in its stalks.

Table 55. The relationship between hybrid and plant part on Anibas farm in 2019. Significant differences between hybrids for the same plant part are shown in bold print.

	%N		Ca		K		Mg		P 177		P 213		S 180		S 181	
Corn,17.461	**1.33**	**a**	79	c	3769	e	1077	de	**3170**	**a**	**3175**	**a**	**1047**	**b**	**1087**	**b**
Corn,FOS 8500	**1.11**	**b**	72	c	3840	e	1003	e	**2810**	**b**	**2816**	**b**	**891**	**c**	**890**	**c**
Stalk,17.461	**0.92**	**c**	3446	a	**14806**	**c**	**1941**	**b**	1460	d	1446	d	**700**	**e**	**762**	**d**
Stalk,FOS 8500	**1.03**	**bc**	3390	a	**17638**	**b**	**2140**	**a**	1947	c	1936	c	**874**	**cd**	**937**	**bc**
Root,17.461	0.62	d	1553	b	**10326**	**d**	**1157**	**d**	325	e	326	e	**726**	**de**	**797**	**cd**
Root,FOS 8500	0.75	d	1407	b	**26487**	**a**	**1701**	**c**	502	e	469	e	**1497**	**a**	**1607**	**a**

	%C		Fe		Al 237		Al 394		Mn		Cu		Zn		Si	
Corn,17.461	**44.4**	**c**	124	b	26	c	25	c	8.71	c	0.50	b	14.6	b	65	b
Corn,FOS 8500	**43.9**	**d**	154	b	26	c	26	c	7.82	c	0.56	b	11.0	b	61	b
Stalk,17.461	44.2	cd	343	a	144	bc	141	bc	46.64	a	10.67	ab	29.5	b	**313**	**a**
Stalk,FOS 8500	44.1	cd	341	a	132	bc	131	bc	48.30	a	12.37	ab	28.3	ab	**101**	**b**
Root,17.461	**47.2**	**a**	435	a	326	a	332	a	25.58	b	15.81	a	63.1	a	147	b
Root,FOS 8500	**44.9**	**b**	440	a	229	ab	235	ab	25.76	b	6.70	ab	23.4	b	169	b

Fertiization and inoculation study on Weiss/Bauer farm in 2019.

The treatments on farm Weiss/Bauer differed from the trial at farm Anibas in that the rotation was corn after corn instead of alfalfa, a starter fertilizer with an unspecified amount of N was applied to all plots, and a slurry application was added to half of the plots. There were significant treatment effects due to hybrid ($p < 0.0001$), and fertilization x hybrid x inoculate ($p = 0.0257$), and possible effects of fertilization x inoculate ($p = 0.0846$).

The fertilization x hybrid x inoculate interaction shown in Table 56 shows that FOS outyielded 17.461 and that inoculation had a negative or positive effect on yield, depending on the level of fertilization and on the hybrid. For FOS 8500 the inoculation had a negative effect at the high level of fertilization, but a positive effect at the low level of fertilization For 17.461 the inoculation effects were not significant.

Table 56. fertilization x hybrid x inoculate interaction grain yield in bu/a for the Weiss/Bauer farm in 2019.

Hybrid	Fertilization	Inoculate	LS Mean	
FOS8500	manure + N	none	194.9	a
FOS8500	manure + N	yes	163.4	bc
FOS8500	N	none	153.5	bcd
FOS8500	N	yes	176.5	b
17. 461	manure + N	none	138.0	cde
17. 461	manure + N	yes	134.7	de
17. 461	N	none	124.7	e
17. 461	N	yes	113.8	e

Means not followed by the same letter are significantly different.

Anibas Farm trials 2020.

The same design was applied in 2020 on the Anibas farm near Arkansaw, WI and on the Beiler farm near Rewey, WI in 2020. During this season we compared the experimental Mandaamin hybrid 15.461 with the commercial FOS hybrid FOS8500. These hybrids both possess a relative maturity rating of 105 days. Results from the analysis of variance were no significant effects of any of the treatments or interactions between them on the grain yield of the corn except for Inoculate x hybrid (p=0.037). The Mandaamin hybrid responded negatively to inoculation while the conventional hybrid responded positively.

Literature cited:

Chang, X.; Kingsley, K.L.; White, J.F. 2021, Chemical interactions at the interface of plant root hair cells and intracellular bacteria. Microorganisms 2021, 9, 1041. https://doi.org/10.3390/microorganisms9051041

Craine, J.M, E.N. Brookshire, M.D. Cramer, N.J. Hasselquist, et al. 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. Plant Soil (2015) 396:1–26.

Favela, A., M.O. Bohn, A.D. Kent. 2021. Maize germplasm chronosequence shows crop breeding history impacts recruitment of the rhizosphere microbiome The ISME Journal 15:2454–2464 https://doi.org/10.1038/s41396-021-00923-z

Goldstein,W.A.,W.Schmidt, H.Burger, M.Messmer, L.M. Pollak, M. E. Smith, M.M. Goodman, F.J. Kutka, and R.C. Pratt. 2012. Maize breeding and field testing for organic farmers. pp. 175-189. In: Organic Crop Breeding. Pub. Wiley-Blackwell, NY.

Goldstein W. 2016. Partnerships between maize and bacteria for nitrogen efficiency and nitrogen fixation. Mandaamin Institute, Elkhorn, Wisconsin; published on the Internet, January, 2016. Bulletin 1. www.mandaamin.org.

Goldstein W., A.A. Jaradat, C. Hurburgh, L.M. Pollak, M. Goodman. 2019. Breeding maize under biodynamic-organic conditions for nutritional value and N efficiency/N_2 fixation. Open Agric. 2019; 4: 322–345.

Micci, A., Q. Zhang, X. Chang, X, K.Kingsley, L. Park, P.Chiaranunt et al. 2022. Nitrogen-transfer symbioses in non-photosynthetic cells of leaves and inflorescence bracts of vascular plants. Paper in review.

White, J.F., K.L. Kingsley, S.K. Verma, K.P. Kowalski. 2018. Rhizophagy cycle: an oxidative process in plants for nutrient extraction from symbiotic microbes. Microorganisms, 6, 95; doi:10.3390/microorganisms6030095 www.mdpi.com/journal/microorganisms

White, J.F., K.L. Kingsley, S. Butterworth, L. Brindisi, J.W. Gatei, et al. 2019a. Seed-vectored microbes: their roles in improving seedling fitness and competitor plant suppression. Chapter 1, page 3-20. In Verma, S.K. and J.F. White (editors). Seed Endophytes, Biology and Biotechnology. Springer Nature Press, Switzerland.

White, J.F., Kingsley, K.L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M.T., Verma, S.K., Gond, S.K. and Kowalski, K.P. 2019b, Review: Endophytic microbes and their potential applications in crop management. Pest. Manag. Sci. 75: 2558-2565. doi:10.1002/ps.5527