

# Better utilization of inorganic nitrogen compared to organic nitrogen by a plant-symbiotic fungal isolate of *Alternaria alternata*

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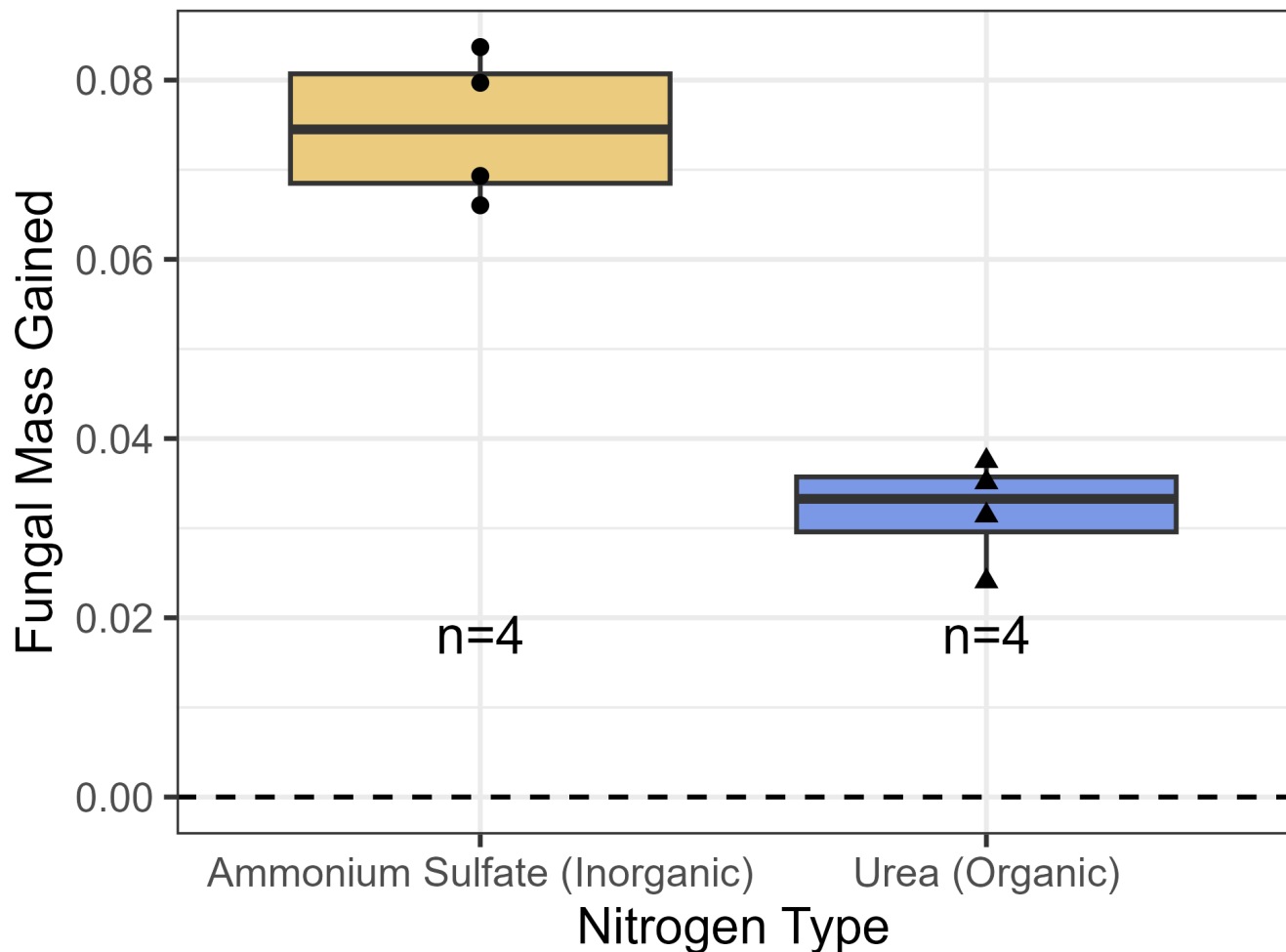
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## Abstract

*Alternaria alternata*, a fungus that causes plant diseases, is also a Dark Septate Endophyte (DSE) that can enhance host plant growth by improving access to soil nutrients like nitrogen. To test the environmental factors influencing this relationship, we explored whether *A. alternata* can utilize both organic and inorganic nitrogen. Our results showed that an *A. alternata* isolate grew 133% larger in an inorganic nitrogen medium than in an organic one. These findings support further research on other DSE taxa and nitrogen forms to better understand fungal nitrogen use.



**Figure 1. Fungal biomass gain when provided with either an inorganic or organic nitrogen source. :**

Fungal biomass growth was significantly higher (133%) in media containing an inorganic nitrogen source compared to an organic nitrogen source (Welch Two Sample t-test; mean<sub>Ammonium Sulfate</sub> = 0.07468; mean<sub>Urea</sub> = 0.03203;  $t(5.3753) = 8.3487$ ,

$p = 0.0003$ ). Points are shaped according to nitrogen type and indicate each replicate in the experiment. Boxplot elements: center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; black points, observations).

## Description

*Alternaria alternata* is a fungus that causes a spot disease in many plant species, which is a concern because of the substantial declines in crop productivity that it can cause (Troncoso-Rojas & Tiznado-Hernández, 2014). *A. alternata* is also considered a Dark Septate Endophyte (DSE), which is a polyphyletic group of ascomycete fungi defined by regular cross-walls among cells (septa) and darkly pigmented hyphae (DeMers, 2022). While many DSE have been categorized as plant pathogens, there is increasing evidence that under certain conditions, these fungi can form mutualisms with plants and improve plant growth through better access to soil resources including nitrogen (Berthelot et al., 2019; Netherway et al., 2024; Schulz & Boyle, 2005). Given that *A. alternata* is a globally distributed species (DeMers, 2022), it is of interest to understand the conditions that are conducive to its function as a mutualist instead of a pathogen so its management in agricultural settings can be improved.

In this experiment, we assess the ability for an *A. alternata* isolate (GenBank Accession Number SUB14593255 germinator\_fung\_10x\_B\_2\_F12 PQ284877) to utilize inorganic and organic forms of nitrogen. It is vital to know if this fungus can use both forms of nitrogen because this will impact which forms of nitrogen are used and monitored in other experiments. For example, stable isotopes of nitrogen are useful in tracing nitrogen movement in plant-fungal symbioses. However, it is unclear which form of nitrogen is best to use for these experiments despite similar work on other groups of fungi (Finlay et al., 1992; Hawkins et al., 2000; He et al., 2003; Newsham, 2011; Upson et al., 2009). Additionally, nitrogen is an important nutrient for plant growth, and its use by different fungi and plants is important in agricultural settings where resources and fungal growth are monitored closely.

In this laboratory experiment, we grew the *A. alternata* isolate in nitrogen-free media and amended it with either organic or inorganic nitrogen. We hypothesized that the fungus would be better able to use organic forms of nitrogen because it is a plant-symbiont which improves plant access to nitrogen, although through unclear mechanisms (Di Martino et al., 2022; Newsham, 2011). In contrast to our expectation, the fungal isolate's growth was 133% higher in the inorganic nitrogen media than in the organic nitrogen media (Figure 1; Welch Two Sample t-test;  $t(5.3753) = 8.3487$ ,  $p = 0.0003$ ). While other sources of inorganic and organic nitrogen should be tested to determine fungal preference between the two types of nitrogen, it is clear that the fungus not only utilized an inorganic nitrogen source, but it also grew better with the inorganic nitrogen source than with the organic nitrogen source in this experiment.

This finding is relevant to a multitude of experiments, including those which aim to use stable isotopes of nitrogen to trace the movement of nitrogen, as the fungus's ability to use the nitrogen source is vital in such a method. Additionally, while more DSE isolates should be tested for their abilities to use inorganic and organic nitrogen, this experiment provides foundational evidence for DSE being able to use inorganic nitrogen sources.

## Methods

We prepared two nitrogen-free media solutions by dissolving 0.0273 grams of Nitrogen free minimal media (MyBioSource Edinburgh) per 1 mL of autoclaved RO water. These solutions were amended with either an organic or an inorganic nitrogen source. For the inorganic N treatment, we added 0.0019 grams of Ammonium Sulfate (Mallinckrodt Chemical Works) per 1 mL of solution. For the organic N treatment, we added 0.0009 grams of Urea (Fisher Scientific U-15) per 1 mL of solution. Ratios were calculated based on the number of N atoms by weight to ensure the same amount of nitrogen was in each treatment. Both solutions were autoclaved and brought to room temperature over 24 hours.

50 mL Erlenmeyer cell culture flasks with metal caps were autoclaved, and 15 mL of either media type was added to each flask with sterile serological pipettes in a sterile biosafety cabinet using aseptic technique. A 4 mm diameter cork borer was used to punch same-sized inoculum plugs from the growing edge of the fungal culture (GenBank Accession Number SUB14593255 germinator\_fung\_10x\_B\_2\_F12 PQ284877), plated two weeks prior on Potato Dextrose Agar. The dry weight of these plugs is approximately 0.0029 g, calculated by averaging the dry weights of four inoculum plugs not used in the experiment. One fresh fungal plug was added to each flask. Flasks were incubated at 19°C shaking at 80 RPM for 7 days. Prior to filtering the fungal contents of each flask, filter papers were labeled and their individual weights recorded. After incubation, the contents of each flask were poured through these filter papers. Flasks were rinsed with autoclaved RO water, and the contents were poured through the pre-weighed filter papers until the visible contents of all flasks were cleared. Once filtering was complete, we placed the folded filter papers upright in a rack in a 59.2°C drying oven for 48 hours. After drying, we weighed each sample. Fungal mass was calculated by subtracting each filter paper weight and the average dry fungal plug weight from the final mass of each filtrate.

## Reagents

Reagent Name	Details	Available From
<i>Alternaria alternata</i> fungus	GenBank Accession Number SUB14593255 germinator_fung_10x_B_2_F12 PQ284877. Isolated from a <i>Sorghum bicolor</i> seed by Dr. Ron Deckert in Dr. Catherine Gehring's laboratory at Northern Arizona University.	Dr. Catherine Gehring's laboratory archive of fungi
Nitrogen free minimal media	Edinburgh minimal media, Nitrogen free. Catalog #MBS652833	MyBioSource
Ammonium Sulfate	Mallinckrodt Chemical Works Ammonium Sulfate	Mallinckrodt Pharmaceuticals
Urea	Fisher Scientific Urea U-15	Fisher Scientific

## Acknowledgements:

## References

- Berthelot, C., Chalot, M., Leyval, C., & Blaudez, D. (2019). From Darkness to Light: Emergence of the Mysterious Dark Septate Endophytes in Plant Growth Promotion and Stress Alleviation. In T. R. Hodkinson, F. M. Doohan, M. J. Saunders, & B. R. Murphy (Eds.), *Endophytes for a Growing World* (1st ed., pp. 143–164). Cambridge University Press. [https://www.cambridge.org/core/product/identifier/9781108607667%23CN-bp-7/type/book\\_part](https://www.cambridge.org/core/product/identifier/9781108607667%23CN-bp-7/type/book_part)
- DeMers M. 2022. *Alternaria alternata* as endophyte and pathogen. *Microbiology* 168: 10.1099/mic.0.001153. DOI: [10.1099/mic.0.001153](https://doi.org/10.1099/mic.0.001153)
- Di Martino C, Torino V, Minotti P, Pietrantonio L, Del Grosso C, Palmieri D, et al., Carfagna. 2022. Mycorrhized Wheat Plants and Nitrogen Assimilation in Coexistence and Antagonism with Spontaneous Colonization of Pathogenic and Saprophytic Fungi in a Soil of Low Fertility. *Plants* 11: 924. DOI: [10.3390/plants11070924](https://doi.org/10.3390/plants11070924)
- Finlay, R. D., Frostegård, Å., & Sonnerfeldt, A. -M. (1992). Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. Ex Loud. *New Phytologist*, 120(1), 105–115. <https://doi.org/10.1111/j.1469-8137.1992.tb01063.x> DOI: [10.1111/j.1469-8137.1992.tb01063.x](https://doi.org/10.1111/j.1469-8137.1992.tb01063.x)
- Hawkins HJ. 2000. . *Plant and Soil* 226: 275-285. DOI: [10.1023/a:1026500810385](https://doi.org/10.1023/a:1026500810385)
- He XH, Critchley C, Bledsoe C. 2003. Nitrogen Transfer Within and Between Plants Through Common Mycorrhizal Networks (CMNs). *Critical Reviews in Plant Sciences* 22: 531-567. DOI: [10.1080/713608315](https://doi.org/10.1080/713608315)
- Netherway T, Bengtsson J, Buegger F, Fritscher J, Oja J, Pritsch K, et al., Bahram. 2024. Pervasive associations between dark septate endophytic fungi with tree root and soil microbiomes across Europe. *Nature Communications* 15: 10.1038/s41467-023-44172-4. DOI: [10.1038/s41467-023-44172-4](https://doi.org/10.1038/s41467-023-44172-4)
- Newsham KK. 2011. A meta-analysis of plant responses to dark septate root endophytes. *New Phytologist* 190: 783-793. DOI: [10.1111/j.1469-8137.2010.03611.x](https://doi.org/10.1111/j.1469-8137.2010.03611.x)
- R Core Team. (2023). *R: A Language and Environment for Statistical Computing* [Computer software]. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Schulz B, Boyle C. 2005. The endophytic continuum. *Mycological Research* 109: 661-686. DOI: [10.1017/S095375620500273X](https://doi.org/10.1017/S095375620500273X)
- Troncoso-Rojas R, Tiznado-Hernández MnE. 2014. *Alternaria alternata* (Black Rot, Black Spot). *Postharvest Decay* : 147-187. DOI: [10.1016/B978-0-12-411552-1.00005-3](https://doi.org/10.1016/B978-0-12-411552-1.00005-3)

Upton R, Read DJ, Newsham KK. 2009. Nitrogen form influences the response of *Deschampsia antarctica* to dark septate root endophytes. *Mycorrhiza* 20: 1-11. DOI: [10.1007/s00572-009-0260-3](https://doi.org/10.1007/s00572-009-0260-3)

**Funding:**

The authors are grateful for the support of the US Department of Energy program in Systems Biology Research to Advance Sustainable Bioenergy Crop Development (DE-FOA-0002214)(Nancy Collins Johnson), the NSF Macrosystems Biology and NEON-Enabled Science Grant (DEB-2017895)(CAG), the Lucking Family Professorship (CAG), the ARCS Foundation (BMB), the Presidential Fellowship Program at NAU (BMB), the Arizona Mushroom Society (BMB), and the Support for Graduate Students program at NAU (BMB).

**Author Contributions:** Beatrice Bock: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing - original draft, writing - review editing. Lexie Curry: conceptualization, data curation, investigation, methodology, writing - review editing. Catherine Gehring: conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing - review editing.

**Reviewed By:**

**History: Received** October 29, 2024

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**Citation:** Bock, B; Curry, L; Gehring, C (2024). Better utilization of inorganic nitrogen compared to organic nitrogen by a plant-symbiotic fungal isolate of *Alternaria alternata*. microPublication Biology.