Dear Editor,

We would like to thank the journal for the opportunity to publish our work, as well as the reviewers for their time and thoughtfulness in responding to our work.

We have gone through each comment below and addressed all to the best of our ability. We believe the manuscript is much stronger now with the added details on the context of nitrogen use in dark septate endophytes and why this could be important in understanding the symbiosis between fungi and plants (Lines 43-47, 68-77). We also hope that the methods have been further clarified regarding the comments of Reviewer #2 (Lines 89-96).

Please let us know if any further edits are suggested.

Kind regards,

Bock et. al.

Reviewer #1

This manuscript, “Better utilization of inorganic nitrogen compared to organic nitrogen by a plant-symbiotic fungal isolate of Alternaria alternata,” demonstrates that a particular isolate of A. alternata produces more biomass when grown in a liquid with ammonium sulfate than with urea as the sole nitrogen source. The methods and results of this manuscript are straightforward and the text is clear. The data support the conclusion. I think this manuscript is appropriate for publication in microPublication Biology, but I also think it could benefit from a little bit more context provided as background and interpretation.

As a reader interested in fungal nitrogen utilization, this manuscript left me wanting to understand how the work done fits into the broader picture. This manuscript’s authors are working in a very interesting system, and they make a good case that this particular fungus is worth investigating. The case is a bit weaker for why the question of which nitrogen sources it uses is a question worth answering (besides that the answer will inform future work in this lab). As a reader, I was left wondering what information there already is about how Alternaria, leaf-spot fungi, or DSE interact with nitrogen. Is the host environment usually N-limited, or can it be limited by other nutrients? Is urea the most abundant organic nitrogen source inside a host? What about ammonium sulfate? Similarly, after the authors give the results, I was hoping for some speculation about why their expectations didn’t pan out. How do ascomycetes in general process nitrogen? Are there lessons from model organisms, such as Neurospora, that might help explain their results?

MicroPublication Biology takes pretty brief manuscripts, so it’s probably not worth it to go into too much depth to answer my questions (or even to answer all of them). But one or two more short paragraphs in the Description section with a few additional citations would really help readers better understand why this result is interesting and where the authors are going next with the “multitude of experiments” they plan to do.

>>> RESPONSE: We thank Reviewer 1 for their time and thoughtfulness put into this review. To address their curiosity about the context for nitrogen utilization in this system and other potential implications, we have added several sentences to the introduction and to the Description regarding this (Lines 38-40, 43-47, 68-77).

Reviewer #2

The paper presents a clear research focus: investigating the nitrogen utilisation preferences of Alternaria alternata, specifically comparing its growth with inorganic nitrogen (ammonium sulfate) versus organic nitrogen (urea) sources. This topic is relevant due to the role of A. alternata as both a plant pathogen and an endophyte, which can influence nitrogen cycling in ecosystems and agricultural settings. The authors make exciting observations that could potentially have significant implications for DSE taxa. However, some aspects of their work can be further clarified.

Replication and Sample Size: The statistical analysis indicates an adequate sample size, but the exact number of replicates is not provided.

>>> RESPONSE: Thank you for pointing out that this was missing in the text. Although replicate information was present in the figure, we have added the replicate numbers to the figure caption and to the text (Lines 22 & 97).

Quantitative Consistency: The nitrogen amounts were balanced by calculating the weight ratios of nitrogen atoms in each source to ensure equivalent nitrogen content. However, it is unclear how nitrogen availability was calculated/confirmed. The authors acknowledge the unexpected outcome - increased growth with inorganic nitrogen - and suggest that this may indicate physiological traits of A. alternata that favour inorganic nitrogen uptake. However, this conclusion cannot be fully supported by the increased biomass alone without precise details regarding the preparation and validation of nitrogen equivalency between treatments and investigation of the organism's genetic makeup. For example, ammonium sulfate often decreases the pH of growth media as fungi metabolise it, potentially affecting growth differently than urea. Although urea is generally neutral in solution, it can undergo hydrolysis and potential volatilisation over time. Also, does A. alternata have urease; if it does not, nitrogen from urea might be less accessible, resulting in decreased biomass.

>>> RESPONSE: Thank you - pH information has been added to the methods section to address these concerns. In addition, we have added information from the literature to clarify the availability of urea to *Alternaria alternata* (Lines 90-92). Previous work (Ismail et al. 2020, referenced in our manuscript) has found that *A. alternata* does produce urease, which means that urea should be a form of nitrogen that is available to *A. alternata.*

Other issues: In the methods section, both ammonium sulfate and urea are referred to as inorganic nitrogen treatment conditions. "Sulphate" and "sulfate" are used to describe the same chemical compound interchangeably.

>>> RESPONSE: Capitalization of all chemical names has been checked and corrected where needed. The typo in the methods section referring to urea as the inorganic nitrogen source has been corrected (Line 89).