Effects of Temperature and Moisture Stress on Nitrous Oxide Production in Agricultural Soil

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ABSTRACT:
As current climate models project continuous increases in temperature and varying precipitation regimes, it is important to know how nitrous oxide production, a highly effective greenhouse gas, will be affected by these different stress factors. Looking at an agricultural corn field in South Deerfield, MA, this study observes the changes in nitrous oxide production fluxes as samples are subjected to varying temperature and moisture levels, as well as the change in nitrous oxide to nitrogen production ratio. Increased production of nitrous oxide, as well as increased nitrogen mineralization and nitrification rates, were found in response in treatments with higher temperature and moisture levels. The highest levels of nitrate were also found in those wetter, hotter incubations; however, there was no visible trend in the concentration of ammonium within the different treatments. There was no visible trend in nitrous oxide to nitrogen gas ratios among the various treatments. The 20 ° incubation showed the highest values of the ratio, but there was no correlation between temperature or moisture and the N₂O to N₂ ratio. Temperature was found to have a positive correlation with nitrification rates, nitrous oxide production, and nitrogen pools in the soil. Moisture level resulted in increased nitrification, mineralization, and nitrous oxide production as well. With no new nitrogen inputs to these systems, the magnitude of these fluxes decreased over time.

Key Words: nitrous oxide production, denitrification, temperature stress, moisture stress, nitrification

INTRODUCTION:
Nitrous oxide (N₂O) is a very minor component of our atmosphere, making up approximately 0.00003% of atmospheric composition. However, it has major significance as a highly effective greenhouse gas. Sixty-five percent of global N₂O emissions originate from soil, while oceans emit thirty percent primarily from estuaries and coastal bodies of water (Smith and Conen, 2004). Nitrous oxide is a natural product of microbial nitrification and denitrification processes; however, the amount of N₂O being emitted into the atmosphere has drastically increased over the past few decades as a result of human activities. These increases have occurred primarily due to anthropogenic activity, which include nitrogen fixation for use in fertilizers and the burning of fossil fuels. Since the industrial era in 1750, N₂O gas has risen by more than 18% and has an estimated linear increase in average production of roughly 0.26% per year (Forster et. al, 2007). Fertilization is the main driver of increasing atmospheric N₂O, and while irrigation and fertilizers enhance plant productivity and organic carbon (OC) storage, application of this nitrogen also emits significant quantities of N₂O and increases soil emissions of other greenhouse gases (Kaye et al., 2004). In addition, N₂O is currently the single most important ozone-depleting emission, and is expected to remain so throughout the 21st century (Ravishankara et. al, 2009). Millions of tons are emitted into the atmosphere every year as a result of human activity, and while carbon dioxide (CO₂) is a widely recognized greenhouse gas, nitrous oxide has roughly 310 times the ability to trap heat in the atmosphere as compared to CO₂.

Nitrogen (N) undergoes numerous processes in soil systems, constantly changing from one form to another. Both organic and inorganic forms of nitrogen are found in soil, and understanding how these different pools interact and the processes by which they enter and leave the soil is important in understanding the factors that affect those processes. Soil organic matter is converted to inorganic forms of nitrogen, such as ammonium (NH₄⁺), through processes such as mineralization and ammonification. Once in an inorganic form, nitrates, nitrites, and nitrous
oxide are formed and released through the process of nitrification. Nitrification rates depend on the metabolism of organisms in the soil and as such are strongly dependent on several environmental variables, such as temperature and moisture changes. Increases in temperature will increase nitrification rates. However, a decrease in temperature below ten degrees Celsius will actually inhibit nitrification, as microbial communities’ cellular metabolism will begin to slow and ultimately stop (Fdz-Polanco et al, 1994). Nitrification rates are positively correlated to increases in soil temperature; however, a negative correlation is shown in response to an increase in water-filled pore space due to increased precipitation (Breuer et al, 2000). Nitrification is an aerobic process, so more soil moisture and precipitation results in more water-filled pore space and less aerobic soil. Therefore, nitrification rates are expected to decrease under conditions of high precipitation. Higher rates of nitrification lead to more biologically available nitrates that can be broken further reduced through denitrification.

While nitrification produces subtle amounts of N₂O, denitrification is the major source of N₂O release into the atmosphere. It is an anaerobic, heterotrophic microbial process that is important in ecosystem nitrogen cycling. Denitrification removes biologically available nitrogen from the environment, producing relatively inert nitrogen gas, thus playing an important role in mitigating increased human release of nitrogen into the environment. The reduction of nitrate to N₂ goes through a step which produces N₂O, some of which escapes reduction and is released to the atmosphere:

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 (g) \quad (1)
\]

We do not fully understand the factors that influence N₂O production in actively denitrifying environments. Previous work has shown that the nitrous oxide to nitrogen gas ratio has increased in agricultural soils as salinity deviates from ambient conditions (Weston, unpublished data). Similarly, Ruyters et al. (2010) have shown that as zinc toxicity levels increased, N₂O reduction rates decreased markedly as nitrous oxide production increased. A recent study on N₂O response to climate treatment has also shown increased nitrous oxide fluxes as soil temperature increased by 3.5 degrees C and moisture decreased from normal standards (Cantarel et al, 2009). Stressing these soil systems from their ambient standards and conditions causes changes to their nutrient cycling and emissions. Together, these results suggest that as stress increases on the denitrifying microbial community, the complete reduction of nitrate to nitrogen gas is short-circuited before the final step, resulting in increased rates of N₂O production. As emissions continue to increase, climate change models have projected temperatures to increase and varying precipitation regimes to continue (Alley et al, 2007). It is important to understand how this nitrogen production will react and how soils will change as the stress on their environment increases.

According to a model of varying temperature and moisture effects on a deciduous forest soil, denitrification is expected to increase at higher temperatures and moisture levels (Troy, unpublished, 2011, Figures 1 and 2). Modeling average temperatures and variations by five degrees Celsius, denitrification showed the highest rates in increased temperature conditions. Two modeled moisture treatments (control and increased precipitation by 20%) show denitrification production rates highest at the increased moisture level, due most likely to a more ideal environment for denitrification created by an increase in volume of anaerobic soils. In these same model runs, nitrification was expected to increase with temperature (Figure 3); however, due to the increase in anaerobic soil, wetter conditions showed a decrease in nitrification rates (Figure 4). Under levels of high moisture the environment will most likely be...
too wet to produce good biologically available nitrates and ammonium. The predictions of denitrification and nitrification fluxes under different conditions that this model shows served as a base for my hypothesis and further experimentation.

In this research, I observed the effects of temperature and moisture stress on nitrous oxide production in an agricultural soil collected from an established corn field in South Deerfield, MA. As stress is increased on these denitrifiers and the further these factors deviate from the soils’ ambient standard, nitrous oxide production is predicted to increase at higher temperatures and increased moisture. Similarly, as temperature and moisture are increased and the stress on these denitrifying communities increases, the nitrogen oxide (N\textsubscript{2}O) to nitrogen (N\textsubscript{2}) ratio is expected to increase, as nitrogen oxide reduction rates will decrease. As the incubation period extends, a decrease in the values of the fluxes in each temperature and moisture treatment is expected, due to no new inputs of nitrogen to the samples. Nitrogen concentrations in the soil, however, are expected to increase over the incubation period, showing highest nitrification and mineralization rates at higher temperatures. An increase with temperature is projected to increase nitrification rates, but once the soils reach anaerobic conditions at higher moisture levels, a decrease in nitrification rates is predicted.

**METHODS:**

**Experimental Design and Sampling.** To assess nitrous oxide production, 27 samples from an agricultural plot at the University of Massachusetts Agricultural Experimental Site at 89 River Road, South Deerfield, MA were collected (Figure 3). The production of nitrous oxide was measured at the field site with five collars in both November and December to get field conditions at different temperatures. Temperature, moisture, and bulk density measurements were also taken at the sampling site. Each soil sample was homogenized and equalized to 18.2 kilograms in a 20-litre plastic bucket and subjected to varying temperature and moisture treatments in order to test the hypothesis. To test rates of denitrification and N\textsubscript{2}O production under various levels and mechanisms of stress, three soil samples in triplicate were prepared with varying amounts of de-ionized (DI) water in order to attain different constant volumetric based moisture levels: 10, 20 and 30% soil moisture. These samples were then placed into 10, 20, and 30 °C incubation chambers, as shown in Figure 6. There were a total of nine treatments: three levels of moisture in triplicate in each of the three temperature incubations. Using a moisture sensor to monitor moisture levels of the buckets, DI was added to the buckets when needed to maintain constant levels.

**Incubations and Treatment.** A 24 day incubation period began on November 14\textsuperscript{th}, 2011, allowing one day of incubation before taking initial measurements. Nitrous oxide production was measured using a laser-based N\textsubscript{2}O/CO analyzer (LGR, Los Gatos Research, Inc., Mountain View, CA; 2010). The chamber connected to the LGR was placed over the samples, each for five minutes. By placing the chamber over the buckets and sealing the sample from outside air, the LGR picked up changes in N\textsubscript{2}O concentrations within the sample space. Start and stop times of measurements were recorded and measurements were made every two to three days in order to get a change in flux over time, stopping incubations on December 9\textsuperscript{th}, 2011. Using Excel, the data from the times recorded was used to calculate the nitrous oxide flux for each bucket. The fluxes of each treatment were then averaged in order to determine the effects of moisture and temperature on these fluxes.
**N₂ Analysis.** In order to measure nitrogen gas in an accurate way, without contamination, nitrous oxide fluxes were measured on a smaller scale and calculations were made to determine the amount of nitrogen gas assumed to have been produced. Because nitrogen gas is such a major component of our atmosphere, it is difficult to measure directly without contamination from atmospheric N₂. Acetylene blocks the last stage of denitrification from nitrous oxide to nitrogen gas. 50 grams of soil from each bucket were sub-sampled into two 500mL mason jars: one control and one treated with 10% by volume acetylene (50 mL). Every jar was kept in the same incubation as the original bucket sample and was assumed to have the same soil moisture as the sample it was taken from. These jars acted as a closed system; they were sealed and measurements were taken at 0, 12, and 24 hours from the initial sealing. Assuming that the production of nitrogen gas was similar in the duplicate jars, the difference between the average of the jars with acetylene and those without was calculated in order to determine how much nitrogen gas was produced from each treatment.

**Nutrient Analysis.** Rates of N mineralization in these soils was measured by examining the change in ammonium and nitrate concentrations between the initial nitrogen concentrations and concentrations after a 24 day incubation period in the different treatments. I performed potassium chloride extractions (KCl) on roughly 10 gram subsamples from the top 0-4 centimeters of each sample bucket, as well as two extractions of the soil initially divided between the 27 buckets (KCL Extraction of Soils for N mineralization, SES, 2011). After shaking these extractions for an hour and a half, they settled overnight and were filtered to undergo further analysis. I determined ammonium and nitrate concentrations by using a Shimadzu 1601 spectrophotometer and Lachat Flow Injection Analyzer, respectively, following the lab protocol (Strickland and Parsons, 1972; LaMotte Hach Method, SES, 2011).

To determine wet to dry ratio of the soil, ten grams of wet soil were weighed and dried at 60°C for 24 hours and re-weighed. This ratio of wet to dry soil was used to determine the weight of dry soil in the extractions and quantify the N mineralization rates per gram dry soil. After determining the soil’s dry to wet ratio, I was able to determine the volume of extractant and moisture in the samples. Using these volumes, I was able to convert the measured ammonium and nitrate concentrations into micrograms of ammonium and nitrate per gram of dry sample. Using the values of the initial soil and the final values from each bucket after the 24 day incubation period, I was able to calculate the net N mineralization and nitrification rates of each treatment in grams N per gram of dry soil per day.

**RESULTS:**

**Nitrous Oxide Production.** For all treatments, nitrous oxide production generally increased with increasing temperature and moisture (Figure 7). Of the two stress variables, temperature had a greater effect on nitrous oxide production than moisture. As temperature increased, there was a clear positive effect in nitrous oxide production. The most nitrous oxide was being produced in the 30°C incubation room, while the least was being produced in the colder, 10°C incubation room. As the moisture level increased within the incubations, nitrous oxide production increased, showing highest levels of production at approximately 30% soil moisture. However, in the 30°C incubation, the highest level of nitrous oxide production was seen at around 20% moisture. These fluxes were greatly affected by these treatments, showing a more positive correlation to temperature variations. Over the twenty four day incubation period, the magnitude of these fluxes all decreased (Figure 8). The measurements from the last day of incubation were much
lower than the fluxes from the initial day of incubation, observed in all nine treatments. My field measurements were very comparable to the data from this experiment, the November readings fitting to the 20°C measurements and the December readings fitting to the 10°C measurements (Figure 7).

**Nitrogen Gas Production.** There was no correlation found between temperature or moisture and the nitrous oxide to nitrogen gas ratio (Figure 9). The 20°C incubation showed the highest values of this ratio, with the 10°C incubation expressing values around zero. The 30°C incubation showed all negative values, showing more nitrous oxide production in the jars without acetylene than those with the addition.

**Nutrient Analysis.** Higher temperature treatments showed higher rates of both net N mineralization and nitrification rates (Table 1). The 30°C incubations showed net N mineralization rates ranging from approximately 1.14-3.21 µg N per gram of dry soil per day, whereas the 10°C incubations showed rates ranging from 0.39 to 0.22 µg N per gram of dry soil per day. The highest nitrification rates were also found in the 30°C incubation, similar to the N mineralization trends. The 30 °C incubation showed daily nitrification rates ranging from 0.90 to 3.16 µg N per gram dry soil per day, increasing with moisture level (Figure 10). The coldest incubation showed a similar trend in nitrification as in net N mineralization, with a disparity between the 20 and 30% moisture levels. The 10°C incubation showed the highest mineralization and nitrification rates at 20% moisture level.

The highest amounts of nitrate were found in higher temperatures; however, there was not a clear trend in ammonium concentrations as a response to temperature. Generally, as moisture increased in each treatment, the ammonium concentrations decreased, while the nitrate concentrations increased. Within each temperature treatment, nitrification and N mineralization rates increased with higher moisture, with the exception of the cold, 10°C incubation. The 20% moisture level showed the highest N mineralization and nitrification rates in the coldest incubation.

**DISCUSSION:**

**Nitrous Oxide Fluxes.** In general, the treatments showed a positive response to higher soil moisture levels (Figure 7). In wetter soils, there is a greater percentage of anaerobic soil which is attractive to the denitrifying communities that need anaerobic conditions to reduce nitrates. In the soils that were subjected to 30% soil moisture, the values of nitrous oxide production were relatively higher than the samples subjected to lower soil moisture levels in the same temperature incubations. However, in the 30°C incubation, the largest rates of nitrous oxide production were found in the soils with approximately 20% moisture, not 30%. It is possible that these soils never fully reached anaerobic soils. In hotter climates, more evaporation is occurring and it’s hard to monitor and keep a constant state of saturation. Because the moisture readings were taken in all of the temperature incubation chambers at the same times, and not more frequently in the 30°C room, it is possible the warmer soil incubation’s soil moistures were experiencing greater fluctuations and did not maintain the monitored percentages for long.

All of the treatments showed positive responses to increased temperature. The incubations at 30°C showed the highest average ranges of nitrous oxide production rates over the 24 day incubation period (Figure 7). The 30°C room showed the highest rates for every moisture level,
with rates decreasing at lower temperatures. Nitrification and denitrification are positively correlated with increases in temperature. At higher temperatures, more nitrogen is being converted from ammonium to nitrates, allowing more nitrates to be made readily available for denitrification.

Similarly, these values of nitrous oxide production decreased over the 24 incubation period as expected (Figure 8). One possible explanation is that there was no new nitrogen being inputted into the soil samples during these incubations, and these soils are accustomed to regular fertilization, as they were collected from an established corn field. With no new nitrogen inputs, there is less organic nitrogen to initially undergo mineralization. Therefore, over time, these values were expected to decrease. Once these soils reach anaerobic conditions, the feedbacks between nitrification and denitrification are not completely understood. They are important to one another, as nitrification provides the substrate for denitrification; however, they function under much different conditions.

\( \text{N}_2 \) Analysis. The nitrous oxide to nitrogen gas ratio showed no correlation to changes in temperature or moisture (Figure 9). This can be explained most likely to a flaw in method. The amount of acetylene added was 10% by volume of the mason jars, 50 mL. This amount could have been too much for these soils, resulting in inhibition of nitrous oxide production as well as an inhibition of further reduction to nitrogen gas. Also, the addition of this gas could have changed the concentration in the jars and therefore altered the readings of nitrous oxide from the LGR. Further studies would have to assess how the acetylene addition affects the nitrous oxide production readings and what volume would be ideal to see any change. Similarly, perhaps the time scale of this experiment could be altered to see how long the acetylene needs to inhibit the soils ability to reduce nitrous oxide to nitrogen gas effectively. It would also be important to do a control jar with just acetylene to see how the LGR reacts to different gases.

Nutrient Analysis. All the treatments showed a general trend in increasing net N mineralization with an increase in moisture. The cold incubation was expected to have the lowest values, as below 10°C nitrification is inhibited altogether. The colder temperatures slow down microbial processes and the cellular metabolism of the living organisms carrying out these processes. The cold incubation was the only incubation that did not follow the positive response to soil moisture increasing with temperature incubation (Table 1). The highest value of mineralization in the cold incubation was found at 20% soil moisture, and the 30% soil moisture sample actually underwent the lowest net N mineralization. This disparity also applied to the net nitrification rates found in each treatment. The cold incubation showed the highest nitrification rates at 20% soil moisture and the lowest at 30%, while both the ambient and warm incubations showed increasing nitrification rates with increase in soil moisture. It is possible that in this incubation chamber the 30% moisture treatment was too anaerobic for nitrification to effectively occur.

The 10°C incubation that showed lower values for its ammonium and nitrate pools in the 30% soil moisture treatments might have been due to the fact that these soils were too wet to produce much biologically available nitrogen. There was not a clear trend in ammonium concentrations in response to temperature. Mineralization rates were highly dominated by nitrification, so as soon as organic nitrogen was converted to ammonium, it was almost immediately processed into nitrates. In both the 20 and 30°C incubations, the highest amount of ammonium was found in the drier soils; whereas, nitrates were found to be greatest in the wetter soils. Wetter soils are undergoing more denitrification, as there is more anaerobic soil available. Therefore, where more
denitrification is occurring, there needs to be more mineralization and nitrification occurring in order to supply denitrification with the necessary amounts of biologically available nitrogen. Higher nitrous oxide production rates are coupled with the higher nitrogen pools and higher rates of mineralization and nitrification. However, it is clear that there is something else going on in these soils, because as time went on the rates of nitrous oxide production decreased, while nitrogen pools continued to increase. It would be interesting to look into what other processes could be causing this increase in nitrogen pools, because denitrification doesn’t seem to be using the available nitrogen. The final mineral pools of nitrogen are reflective of what is left over after these processes take place and represents the nitrogen in the field that is available for possible export with water and leaching in the soil.

Nitrification was expected to increase with temperature increase, but also decrease at a heightened moisture level, due to increase in anaerobic soil volume. This was seen in only the 10°C incubations (Figure 10). The response to moisture in the 20 and 30°C incubations was different from expected, showing an increase with every increase in moisture level. The soils that showed further increases in nitrification in 30% moisture must have not reached fully anaerobic conditions because if they had then no nitrification would have been able to occur. It’s hard to gauge how these processes will react when two treatments are coupled together. Denitrification and nitrification respond differently to increased moisture but similarly to increased temperature. One process will inevitably have an effect on the other. In a further study, it would be important to see the change in these processes when anaerobic conditions were definitely reached.

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LITERATURE CITED:


FIGURES:
Figure 1. Modeled effects of varying temperature stress on denitrification rates.
Figure 2. Modeled effects of varying moisture stress on denitrification rates.
Figure 3. Modeled effects of varying temperature stress on nitrification rates.
Figure 4. Modeled effects of varying moisture stress on nitrification rates.
**Figure 5.** Map of sampling site, U.Mass Agricultural Research Site.

**Figure 6.** Experimental design and sample incubations for nitrous oxide measurements.

**Figure 7.** Average nitrous oxide fluxes of the various treatments over the 24 day incubation period, with error bars depicting standard deviation.

**Figure 8.** Nitrous oxide fluxes in the various temperature incubations, comparing initial incubation measurements and final incubation measurements.

**Figure 9.** The nitrous oxide to nitrogen gas ratio calculated from the difference between nitrous oxide measurements of control and acetylene treated duplicate sub-samples of the different temperature and moisture treatments.

**Figure 10.** Daily net nitrification rates of the samples in different temperature incubations, varying by soil moisture.

**TABLES:**

**Table 1.** Nitrate and ammonium concentrations of the various treatments with calculated values of net N mineralization and nitrification rates.
Figure 1. Modeled effects of varying temperature stress on denitrification rates of a deciduous forest. Temperature drivers represent the recorded average daily temperatures, variations created by increasing and decreasing the average by 5°C (Troy unpublished, 2011). As temperature is increased by 5°C, denitrification rates increase. The lowest denitrification rates can be found in the treatment where temperature was decreased by 5°C.
Figure 2. Modeled effects of moisture stress on denitrification rates. Moisture treatments are representative of the forest’s average annual precipitation and an increase of 20% precipitation (Troy unpublished, 2011). With higher precipitation, higher rates of denitrification are expected due to a change in the volume of anaerobic soil.
Figure 3. Modeled effects of stress from varying temperature on nitrification rates in a forest system (Troy unpublished, 2011). In warmer temperatures, nitrification rates are predicted to increase. The lowest nitrification values were seen in the treatment where temperature was decreased by 5°C from the control.
Figure 4. Modeled stress effects of varying moisture on a forest system’s nitrification rates (Troy unpublished, 2011). Nitrification rates are expected to decrease with increased precipitation, due most likely to the increased volume of anaerobic soil.
**Figure 5.** Map of sampling site, U.Mass Agricultural Research Site: 89 River Road, South Deerfield, MA 01373; arrow indicating North and scale to size.
Figure 6. Experimental design and sampling incubations. Circles represent buckets in each treatment. Three temperature treatments: 10°C incubation chamber, 20°C (room temperature) incubation, and 30°C incubation chamber. The three moisture treatments were 10, 20, and 30% soil moisture, shown in the different color buckets.
Figure 7. Nitrous oxide fluxes of the various treatments, with error bars depicting standard error. An exponential trend line was fit to each of the different treatments, all showing positive correlations to temperature increase. Field conditions are represented by the stars. The yellow star represents the November field readings, $3.385 \times 10^{-5}$ nmol N$_2$O m$^{-2}$ s$^{-1}$ at 19°C and 22% soil moisture. The purple star represents how the December field readings at colder temperatures relate to the samples in lab, $2.647 \times 10^{-6}$ nmol N$_2$O m$^{-2}$ s$^{-1}$.
Figure 8. Nitrous oxide fluxes for 30°C incubation and varying moisture treatments over the incubation period. The nitrous oxide production decreases as the incubation period extends.
Figure 9. The nitrous oxide to nitrogen gas ratio calculated from the difference between nitrous oxide measurements of control and acetylene treated duplicate sub-samples of the different temperature and moisture treatments. No correlation to temperature or moisture seen in this experiment, due mainly to a flaw in methods.
Figure 10. Daily net nitrification rates of the samples in different temperature incubations, varying by soil moisture. Nitrification rates of the various moisture treatments showed very strong positive correlations to an increase in temperature. Similarly, as moisture increased to 30%, nitrification rates also showed a general increase.
Table 1. Average nitrate and ammonium concentrations of the various treatments with calculated values of daily net N mineralization and nitrification rates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NH$_4^+$ Pool</th>
<th>NO$_3^-$ Pool</th>
<th>Net N Mineralization</th>
<th>Net Nitrification Rate</th>
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<tr>
<td>10°C, 10%</td>
<td>1.85</td>
<td>10.43</td>
<td>0.39</td>
<td>0.36</td>
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<td>4.10</td>
<td>14.80</td>
<td>0.67</td>
<td>0.54</td>
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<td>6.02</td>
<td>0.22</td>
<td>0.17</td>
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<tr>
<td>20°C, 10%</td>
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<td>16.73</td>
<td>0.67</td>
<td>0.62</td>
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<td>20°C, 20%</td>
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<td>30.24</td>
<td>1.20</td>
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<tr>
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<td>0.94</td>
<td>31.95</td>
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<tr>
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<td>3.21</td>
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