Environmental tradeoffs between nutrient recycling and greenhouse gases emissions in an integrated aquaculture-agriculture system

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Supplementary Methods:

Estimation of N₂O production pathway

The N₂O emissions were assigned to different pathways based on the environmental requirements of each pathway (i.e. oxygen, N and C available in the system; Table S1) and the similarity of root zone oxygen concentration (aeration status) and NO₃⁻ concentration for all treatments for the whole season. The contribution of the irrigation water degassing to the total emissions was considered to be the total amount of N₂O dissolved above the concentration of N₂O-N at equilibrium with the atmospheric concentration (i.e. 0.27 µg L⁻¹) per amount of irrigated water over time (Table 1 in main text). For the AN treatment, all N₂O emissions beyond the degassing were considered to be from denitrification as that is the only plausible pathway for NO₃⁻ to be transformed to N₂O within environmental conditions constrains (Table S1). Anaerobic or microaerophilic conditions required for the denitrification potentially existed lower down in the root zone where the soil was saturated, as the bottom boundary condition was atmospheric. It was assumed that the same amount of denitrification taking place in the AN treatment could also take place in the FN+ treatment, although it could also be less due to the lower availability of C (see the Carbon addition experiments section, Table 1 in the main text and Table S1). The remainder of the N₂O emissions in the FN+ treatment were assumed to occur via autotrophic nitrification, potentially including incomplete hydroxylamine oxidation and nitrifier denitrification. The denitrification in the AN+ treatment was assumed to be the same as that of the AN treatment. As similar amounts of NH₄⁺ and NO₃⁻ were present in the AN+ and FN+ treatments, autotrophic nitrifier pathways were assumed to account for the same amount of N₂O emissions in these treatments. The N₂O emissions, beyond what could be accounted for by degassing, denitrification, and the autotrophic nitrifier pathways in the AN+ treatment, were presumed to occur via the heterotrophic nitrification pathway (Table S1).

Supplemental Results and Discussion:

N₂O fluxes over time and plant growth curve

Over the length of the growth season more than 450 individual measurements of soil N₂O fluxes were performed. The fluxes exhibited very high temporal variability (Figure S1), explained by post-fertigation peaks with a rapid decline in emissions with time (Figure 3 in the main text and Figure S1).
The growth curve for the different parts of the cucumber plant can be seen in Figure S2. Differences in the growth rate and yield between the treatments were not found (Figure 1 in the main text and Figure S2). The identical growth rates and yields were expected as the same amount of water and fertilizers was applied to the crops (Table 1 and Figure 1 in main text and Table S1).

**Estimation of N₂O production pathway**

Degassing contributed between 10 and 4% to the total N₂O emitted from the AN and AN+ systems, respectively, and none for FN+ (Figure S3). The anaerobic conditions required for denitrification were not prevalent in the upper soil layer, as the oxygen concentration there fluctuated between 15 and 20%, with an average value for all treatments of 18.5% (± 0.8%), but did exist in the lower water-saturated soil layer. As the TOC and the level of anaerobicity in the AN and AN+ treatments was similar (Table S1) but the N₂O emissions of AN+ were much higher, the limit of denitrification in AN was assumed to be due to the lack of anaerobic conditions and not due to limitation in C. According to Table S1, autotrophic nitrification was the only N₂O emission pathway for the FN+ treatment. However, any C that becomes available in the root zone, from root exudates, for example, could lead to denitrification. A similarity in root zone aeration would limit the denitrification pathway to producing no more N₂O than in the AN treatment, so that the rest of the N₂O emissions from FN+ would be due to an autotrophic nitrification pathway. The assumption that this autotrophic nitrification pathway would account for the same amount in both the AN+ and FN+ treatments is based on the reasoning that the differences in available C do not affect this pathway. The remaining N₂O emissions from the AN+ treatment are attributed to heterotrophic nitrification and the potential aerobic denitrification associated with it. According to this reasoning, heterotrophic nitrification accounts for more N₂O emissions than does autotrophic nitrification, a phenomenon that has been demonstrated to be possible in pure culture studies. The substrate for heterotrophic nitrification is thought to be mineral NH₄⁺, as the availability of NH₄⁺ was significantly higher in the AN+ treatment than in the AN treatment, while the TOC levels were similar. Heterotrophic nitrification has often been associated with acidic conditions which limit autotrophic nitrification, but acidic conditions are not obligatory for heterotrophic nitrifiers. Heterotrophic nitrification was found in different environmental niches and using wide variety of C and N sources. As anammox does not produce N₂O it was not considered here. Chemodenitrification was also not expected to play a role as the pH was around 6.5.
Figure S1. The N₂O emission measurements per treatment throughout the growing season (top left) and the measurements carried out on DAT 14, 22 and 36 with the irrigation time marked with a black arrow. The treatments are the same color as in the top left legend, and the 6 replicates per treatment are distinguished by the shapes listed in the legend of DAT 22.
Figure S2. Growth curve of the cucumber plants split into leaves, stems and fruit. The treatments are marked with □ for AN, ○ for AN+ and Δ for FN+.
**Figure S3.** Cumulative N₂O emissions calculated by means of the exponential model with the parameters listed in Table 2 (main text), and an estimation of the different pathways contributing to this production for each treatment according to Table S1. The hashed colors indicate ambiguity with regard to which pathway the N₂O emission should be assigned.
Table S1. Pathways of N₂O production followed by environmental condition required for the pathway to take place in the integrated aquacultural system. Plus signs are present in case the requirement is met. The shading indicates treatments in which all requirements are met for a pathways that likely contribute to the N₂O emissions of that treatment.

| N₂O emission pathway         | Pathway requirement* | AN | AN+ | FN+ |
|------------------------------|----------------------|----|-----|-----|
| Abiotic degassing            | [N₂O] > 0.27 µg L⁻¹ | +  |     |     |
| Heterotrophic denitrification| Anaerobic conditions | +  |     |     |
|                              | Carbon present       | +  |     |     |
|                              | NO₃⁻ present         | +  |     |     |
| Autotrophic nitrification    | NH₄⁺ present         | -  |     |     |
|                              | Aerobic conditions   | +  |     |     |
| Heterotrophic nitrification  | Aerobic conditions   | +  |     |     |
|                              | NH₄⁺ present         | -  |     |     |
|                              | Carbon present       | +  |     |     |

* Specific pathway was assumed to be plausible only if all environment conditions for the pathway were met within the treatment.
**Table S2.** Average measured concentrations of nutrients other than nitrogen which were added to each of the treatments daily ($n=2$).

| Nutrient | AN | AN+ $mg L^{-1}$ | FN+ |
|----------|----|-----------------|-----|
| P        | 18 | 16              | 20  |
| K        | 212| 193             | 135 |
| Ca       | 167| 158             | 177 |
| Mg       | 95 | 94              | 90  |
| SO$_4$   | 713| 671             | 592 |
| Na       | 303| 294             | 244 |
| Cl       | 470| 470             | 460 |
| Cu       | 0.16| 0.12           | 0.12 |
| B        | 0.53| 0.55           | 0.36 |
| Fe       | 1.53| 1.02           | 1.14 |
| Mn       | 0.77| 0.64           | 0.76 |
| Zn       | 0.75| 0.70           | 0.79 |
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