Identification of the fatty acid and the fatty acid methyl ester as the new nitrification inhibitors

The tropical pasture grass, *Brachiaria humidicola* (Rendle) Schweick, produces nitrification inhibitory compounds (termed biological nitrification inhibitors or BNIs) in its shoot and root tissues, and releases BNIs from its roots. During this study, two BNI compounds were isolated and identified from the shoot tissue of *B. humidicola* using activity-guided fractionation.

The BNI compounds in the shoot tissue were identified as linoleic acid (LA) and linolenic acid (LN) using authentic chemicals (ED_{80} 16.0 μg ml^{-1} for both LA and LN) for verification (Fig. 1). None of the other tested free fatty acids namely stearic acid, oleic acid, arachidonic acid, and vaccenic acid showed any inhibitory effects on nitrification. Among the fatty acid methyl esters (FAME) evaluated [methyl oleate, methyl linoleate (LA-ME) and methyl linolenate (LN-ME)], only LA-ME showed any inhibitory effect (ED_{80} 8.0 μg ml^{-1}) (Figs. 1, 2). The inhibitory effect of LA, LN and LA-ME on soil nitrification was stable for 120 days at 20ºC (Fig. 3). Soil treated with LA, LN and LA-ME showed a very low accumulation of NO_3^- and the maintenance of soil inorganic N in the NH_4^+ form (Fig. 3). The inhibitory effect of LA-ME on soil nitrification was greater than that of LA, LN or nitrapyrin (commercial nitrification inhibitor) (Fig. 3). Both LA and LN suppressed soil nitrifier activity by blocking AMO (ammonia monooxygenase) and HAO (hydroxylamine oxidoreductase) enzymatic pathways in *Nitrosomonas europaea*. Commercial nitrification inhibitors (such as nitrapyrin or dicyandiamide (DCD)) are not effective (nitrapyrin is volatile at temperatures >5C, thus is not effective in tropical environments; DCD is highly soluble in water, thus leaches out of fertilizer zone, thus is not effective in field environments) in tropical environments, thus are not adopted for production agriculture in tropics.

Since LA, LN and LA-ME can be produced from vegetable oils such as soybean, flax or sunflower and are more effective and stable (these compounds are bound to the soil thus do not leach out from the point of application to the fertilizer zone, which is in addition to their stability at tropical temperatures) in tropical soils, they have the potential for use as nitrification inhibitors in production agriculture.

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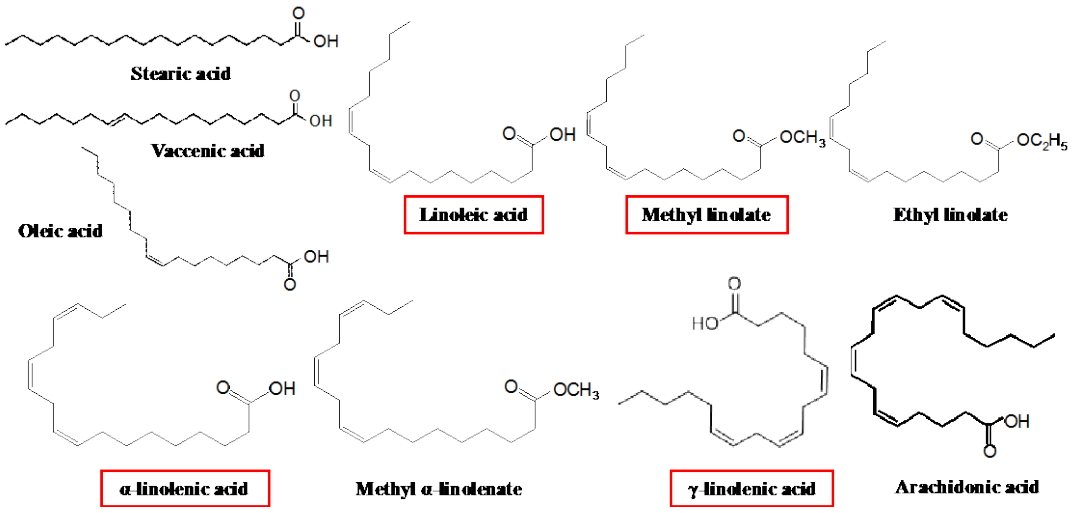

Fig. 1. Structure formulae of various fatty acid and fatty acid ester. Substance with an enclosure has nitrification inhibitory activity.

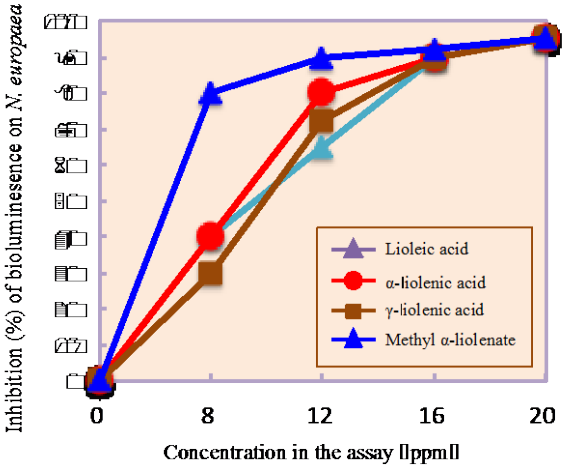

Fig. 2. Relative effectiveness of substances in inhibiting *Nitrosomonas europaea* activity in an *in vitro* assay.

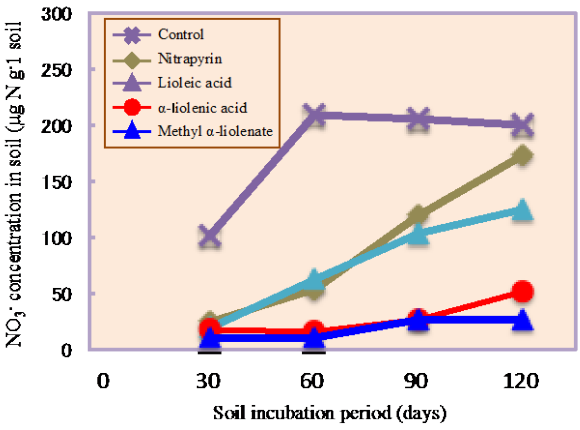

Fig. 3. Relative stability of the inhibitory effects on soil nitrification from linoleic acid (1,000 μg g^{-1} soil), linolenic acid (1,000 μg g^{-1} soil) , methyl linoleate (1,000 μg g^{-1} soil) and nitrapyrin (4.5 μg g^{-1} soil) during 120-day incubation period at 20°C.