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Quantification of Carbon Flow by Stable Isotope Fractionation in Methanogenic Rice Field Soils

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Wetlands are an important source for the greenhouse gas methane being produced by acetoclastic and hydrogenotrophic methanogenesis. Fractionation of $^{13}C/^{12}C$ can in principle be used to quantify the relative contribution of these pathways, but our knowledge of isotopic fractionation during reduction of CO_2 and turnover of acetate in methanogenic environments is still scarce. Therefore, we measured carbon isotope signatures in two samples of anoxic Italian rice field soils, one with high and one with low content of organic matter. Both soils were incubated with and without methyl fluoride, a specific inhibitor of acetoclastic methanogenesis. In the presence of methyl fluoride only hydrogenotrophic methanogenesis operated, thus allowing the determination of its fractionation factor. In addition acetate accumulated, thus allowing determination of the isotopic signature of the fermentatively produced acetate. Using these data it was possible to quantify the relative contribution of the two methanogenic pathways to total methane production. In the high-organic matter soil, the contribution of acetoclastic methanogenesis to total CH_4 production transiently increased due to increasing acetate concentrations early after flooding. In the low-organic matter soil, methanogenesis from H_2/CO_2 was clearly higher than theoretically expected. Furthermore, isotope fractionation of hydrogenotrophic methanogenesis indicated that the *in situ* energy status of methanogens strongly depended on the availability of organic carbon in the rice field soil system. Collectively, our data show that the study of isotopic fractionation in methanogenic environments allows a deeper insight into the ongoing processes, which may be quite different in the same ecosystem with different content

of degradable organic matter.