EFFECTS OF DIFFERENT METHODS OF ACID HYDROLYSIS ON THE NITROGEN DISTRIBUTION IN TWO SOILS

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SUMMARY
An inorganic (Bainsville) and an organic (Farnham) soil were hydrolyzed by continuous and stepwise hydrolysis with hot 3 N HCl for 1, 2, 3, 4, 11, 15, 18 and 24 h and continuously with hot 6 N HCl for 24 h. The following nitrogen forms were determined: total N, hydrolyzable-N, amino acid-N, amino sugar-N and ammonia-N. Proportions of 'unknown' N were computed from these data.

Continuous hydrolysis with 3 N HCl yielded more amino acid-N and less 'unknown' N than did stepwise hydrolysis with the same acid strength. But continuous hydrolysis for 24 h with 6 N HCl produced more amino acid-N and less 'unknown' N than did hydrolysis with 3 N HCl by either method. It was estimated that 33 and 54% of the total N in the inorganic and organic soil, respectively, was protein-N. The 'unknown' N in the inorganic and organic soil constituted 51 and 37% of the total N, respectively. From our work it appears that the 'unknown' N is not proteinaceous. It can be readily degraded chemically and microbiologically to NH₃ and N-gases. More attention needs to be given to identifying the 'unknown' N which constitutes a large portion of the total soil-N. A more adequate knowledge of the chemical constitution of the 'unknown' soil N may lead to the development of technologies that will make more efficient use of the N in soils.

INTRODUCTION
Recent estimates⁸ show that between 86 and 89% of the total N in soils from widely differing climatic zones is hydrolyzable by hot 6 N HCl. Amino acid-N constitutes between 33 and 41% of the total N, amino sugar-N between 5 and 7%, and between 18 and 32% is released as ammonia. Some of the latter originates from the decomposition of amides, hydroxyamino and other amino acids, amino sugars, the deamination of purines and pyrimidines, and the release of fixed ammonium from clay minerals. Thus, between 41 and 46% of the total soil nitrogen has so far not been identified. This fraction will be referred to in this paper as 'unknown' nitrogen. Between 5 and 16% of the latter may occur in

Ms. 4576
inorganic soils as nucleic acid bases, but no information is at this time available on the identities of the major constituents of the 'unknown' N fraction.

In a recent communication that is of considerable relevance to the subject under discussion, Janel et al. reported on effects of two types of hot acid hydrolysis (continuous vs. stepwise) on the nitrogen distribution in litters and a soil. Continuous hydrolysis refers to hydrolyzing samples for differing periods of time (1, 2, 4, . . . 48 h) and separating supernatants from insoluble residues at the end of each hydrolysis period. Stepwise hydrolysis, on the other hand, involves hydrolyzing sequentially the same sample for 1 h, removing the supernatant, then hydrolyzing the residue for 2, 4, 8 . . . up to 48 h, after separating the supernatant at the end of each hydrolysis period. Janel et al. observed the following advantages of stepwise over continuous hydrolysis: percentages of hydrolyzable-N and amino-N increased with time of hydrolysis but only small changes in hydrolyzable combined-N and NH₃-N were observed. These findings were interpreted to mean that amino acids were gradually liberated from an insoluble matrix and that under these conditions degradation of amino compounds by deamination was minimal. Some of their data are of special interest. For example, with three litters under beech overlying a humo-ferric Podzol, continuous hydrolysis for 48 h with 3 N HCl showed that 40-46% of the total N was amino-N, whereas stepwise hydrolysis for the same length of time and with the same acid strength indicated 80-82% amino-N. Similarly, continuous hydrolysis of a soil sample, taken from the A₂ horizon underlying the litters described above, showed that 46% of the total N was amino-N, while stepwise hydrolysis gave 52% amino-N. Janel et al. believe that continuous or prolonged hot acid hydrolysis of soils and soil organic matter gives rise to secondary interactions of N-compounds with polysaccharides and polyphenols that are also liberated at the same time. These reactions result in deamination and the formation of insoluble products. By contrast, these reactions do not occur when stepwise hot acid hydrolysis is used. Thus, more realistic assessments of the nitrogen distribution are obtained with the latter method. From the work of Janel et al. it appeared that a large portion of the 'unknown' soil-N consisted of proteinaceous materials and that their detection had escaped earlier workers who had used almost exclusively continuous hot acid hydrolysis. To test this hypothesis, we determined the N-distribution in an inorganic and an organic soil after continuous and stepwise hydrolysis with 3 N HCl. We then compared the results obtained with each other and with those resulting from the hydrolysis with 6 N HCl (the most widely used method) of the same samples. From the data obtained, proportions of 'unknown' N were computed. Thus, our main objective was to uncover whether peptides or proteins were significant components of the 'unknown' soil N.