for publication. Several of the Commissioners objected to the inclusion of this vote with the Declaration because they did not consider it official, and this presumably forms the basis for the statement in Mayr *et al.* (*1*) concerning the vote. However, the issue was submitted a second time to the Commission, asking if they approved the publication of the vote, and the ballot on this question was 17 to 5 in favor.

The comments of the Commissioners as published show a deep schism within the Commission on the validity of the actions taken on this Declaration, and we consider it unfortunate that Mayr and his colleagues have not made this schism clear in their statement because it will be read and acted upon by many who have no access to the Declaration itself. If the opinions of the majority of the Commissioners are to be accepted, one cannot accept the minority opinion expressed by Mayr *et al.*

Mayr *et al.* state, as if à fait accompli, that the Commission cannot repeal Art. 23(b). However, in the history following Declaration 43, it is clear that the legal adviser to the Commission, the Secretary, and some Commissioners do believe that the Commission has the authority to delete or suspend parts of the Code, such as Art. 23(b).

The important question for the working taxonomist is, if the Commission does have this authority, did they in fact repeal or suspend Art. 23(b). As defined by Art. 78 of the Code, a Declaration is a provisional amendment to the Code. It is to be issued by the Commission in a case that "involves a situation that is not properly or completely covered by the Code," and this Declaration remains in force until the next succeeding Congress ratifies or rejects it. We believe that the Commission did suspend Art. 23(b) until the next International Congress of Zoology this summer and that zoologists who are seeking to preserve well-established names must apply to the Commission to preserve them under the plenary powers.

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4 AUGUST 1972

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3 January 1972; revised 23 March 1972

The history of the Statute of Limitation is long and tortuous, and of no interest to the working zoologist. This is the reason why we restricted our note (*1*) to the undisputed facts. The publication of the "repeal" of Art. 23(b) (Declaration 43) was based on a misunderstanding by the Secretary of the Commission, and he was asked by the Acting President of the Commission to withdraw it. Pressure of work seems to have prevented him up to now from doing so by publication.

In the meantime the Council of the Commission together with an ad hoc Committee on the Constitution of the Commission met in London (13–15 June) and confirmed that the provisionally adopted wording of Art. 23(b) (*1*) be submitted to the 17th International Congress of Zoology at Monaco for ratification (that is, inclusion in the Code or rejection). None of those present at the meeting (including the Secretary) expressed the opinion that the article was repealed. Indeed it has been uninterruptedly in force, in one version or another, since the present code was published (1961). It is regrettable that Collette, Cohen, and Peters have insisted in publishing their confusing statement even though they were informed about the true facts of the case.

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Use of Variations in Natural Nitrogen Isotope Abundance for Environmental Studies: A Questionable Approach

Kohl, Shearer, and Commoner (*1*) have published values for the alleged contribution of fertilizer nitrogen to nitrate levels of the Sangamon River and Lake Decatur, Illinois. Their data, which are being used to influence proposed legislation to regulate agricultural use of nitrogen fertilizers, were obtained by a method based on slight differences in the natural isotopic composition of soil nitrogen, atmospheric nitrogen, and fertilizer nitrogen. Our experience in the use of isotopes in soil research causes us to question the ability of their method to produce valid quantitative information concerning the sources of nitrate in surface waters.

Kohl *et al.* made three principal sets of measurements: (i) the ^{15}N concentrations of nitrogen fertilizers, (ii) the total amounts and ^{15}N concentrations of nitrate nitrogen in drain-tile waters, and (iii) the ^{15}N concentration of nitrate derived from soil incubated in the laboratory. They expressed their ^{15}N data in terms of $\delta^{15}N$ units, a calculated value where one unit is equivalent to 0.0004 atom percent ^{15}N. The maximum average difference in ^{15}N concentration among the samples they studied was 0.0040 ± 0.0004 atom percent, corresponding to 10.0 ± 1.0 $\delta^{15}N$ units. Using measurements (i) and (ii) to obtain reference values, Kohl *et al.* calculated the fractional contributions of soil and fertilizer nitrogen to nitrate in surface waters from the results of measurement (ii). Even if analytic measurements can be made with precision over this extremely narrow range of detection, we question the validity of the data and their interpretation.

First, we question whether the value +3, which they used for $\delta^{15}N$, is representative of the ^{15}N concentration of fertilizer nitrogen after its addition to soil. Their use of this value presupposes that fertilizer nitrogen enters into and is released from the soil organic complex without a change in its isotopic identity, or else that all fertilizer-derived nitrate is formed from the fertilizer directly. Neither of these assumptions is valid.

An indeterminate amount of fertilizer nitrogen (primarily in the ammonium form) mixes with nitrogen in the soil organic complex before it is biologically oxidized to nitrate and loses its identity. Therefore, their value for fertilizer nitrogen had a probable average value in soil other than $\delta^{15}N = +3$. Further, in using this value as a reference point, Kohl *et al.* assume that the ^{15}N concentration of nitrate derived from fertilizer is identical to that of

nitrogen in fertilizer. We question the validity of this assumption.

The second reference value used by Kohl *et al.*, that for soil-derived nitrate ($\delta^{15}N = +13$), was obtained from samples of virgin soil brought into the laboratory from fields (no further description was given) in the study region. The use of virgin soil as being representative of cultivated soils is not valid. Nitrogen transformations in virgin soils are quantitatively, and perhaps qualitatively, different from those in their cultivated counterparts. Further, Kohl *et al.* did not specify the length of their laboratory incubation periods or the amounts of nitrate obtained thereby. These are important considerations in evaluating nitrogen isotope equilibrations. We judge that their value $\delta^{15}N = +13$ for soil-derived nitrate was obtained after prolonged incubation. Most of the nitrate produced from soil organic matter is derived from the readily mineralizable fraction, and mineralization of this fraction is best characterized by a short-term incubation procedure. Cheng, Bremner, and Edwards (*2*) found that $\delta^{15}N$ values for nitrate derived from soils that were incubated for 2 weeks in the laboratory were consistently one-third to one-half as large as those for total nitrogen in the respective soils. Therefore, it appears to us that the reference value for soil-derived nitrate should have an average $\delta^{15}N$ value lower than +13, and that the value should depend on the method and the period of incubation. Also, the data of Kohl *et al.* do not show the extent of variation that can be expected among their soil samples, which were taken as being representative of an extensive soil area.

Kohl *et al.* then used a linear interpolation between what we consider to be two invalid reference values to interpret data obtained from nitrate in drainage waters, a river, and a lake. They assumed that "the nitrate mineralized from the soils of the region is accurately characterized by the measurements made at one point in the 900-square-mile watershed." They assumed that this must be so because data obtained from drainage waters appeared to fit a regression line drawn through the two reference points. We find completely unreasonable the assumption that one survey source (about 1 square mile) can represent 900 square miles of this soil area. A soil classification study of this area shows at least 50 soil series.

Another assumption is that soil organic matter and fertilizer are the only significant sources of nitrate in the drainage water. Kohl *et al.* admitted that rain and symbiotic nitrogen fixation could add about 20 to 27 percent as much nitrogen annually as was added to soil as fertilizer. We consider these values to be minimal, but even so, they represent a significant nitrogen input that should not be ignored. These potential nonfertilizer sources of nitrate have $\delta^{15}N$ values similar to that of fertilizer and therefore cannot be distinguished from fertilizer on the basis of nitrogen isotope compositions.

Isotope fractionation introduces further difficulties in the interpretation of data obtained from biological studies for which nitrogen isotope measurements must be made within extremely narrow limits of detection (*3*). Such fractionation is the cause of variation in ^{15}N concentrations among soils, within a soil profile, and among different nitrogen fractions of a soil (*2*). It is difficult to evaluate and make quantitative corrections for the effect of biological, chemical, and physical isotope fractionation processes in soils on the ^{15}N concentrations of soil isolates.

The main argument of Kohl *et al.* in support of their method stems from the inverse relationship that they observed between nitrate concentrations in waters and the corresponding $\delta^{15}N$ values (figure 1 in their report). They inferred, but did not prove, that where the nitrate concentration in water is high, it is the presence of fertilizer nitrogen that lowers the ^{15}N concentration of that nitrate. The apparent inverse relationship is interesting and merits consideration, but additional data with valid controls are needed before we can evaluate their interpretation or consider alternative explanations.

We object to the manner in which the plot of nitrate versus ^{15}N concentrations is used. On a regression line constructed from data obtained for waters apparently drained from an area of about 1 square mile (as already discussed), Kohl *et al.* plotted information obtained for waters emerging from an area of at least 900 square miles. They erroneously assumed that the soils of the entire study area are relatively uniform.

As scientists active in soil and fertilizer nitrogen research, we are conscious of the potential impact on the environment that increased use of nitrogen fertilizers may have. We deplore indiscriminate and excessive use of nitrogen fertilizer. However, we are also conscious of our responsibility to provide public information that is accurate and unambiguous. Because we believe that the method of Kohl *et al.* is one of unproved reliability, we consider it our responsibility to discuss this question in an open forum.

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2 February 1972

Hauck *et al.* (*1*) make essentially four points which are technical in content. These points are related to: (i) the loss of identity of fertilizer nitrogen as it mixes with the pool of organic nitrogen, (ii) the relative uniformity of soils of the region, (iii) the effect of isotopic fractionation, and (iv) the appropriate length of an incubation ex-

periment to determine the properties of the mineralizable fraction of the soil organic matter.

1) We agree that "an indeterminate amount of fertilizer nitrogen (primarily in the ammonium form) mixes with nitrogen in the soil organic complex before it is biologically oxidized to nitrate and loses its identity" (*1*). This loss of identity of fertilizer nitrogen certainly introduces an error in the estimate of its contribution to the nitrate found in surface waters. Specifically, this effect leads to an underestimation of the contribution of fertilizer nitrogen. Consider, for example, simple exchange of fertilizer nitrogen for soil organic nitrogen. In this case, the presence in drainage water of nitrate nitrogen of soil organic origin is due to the addition of the fertilizer nitrogen. However, in our system of accounting such nitrogen atoms are charged to soil nitrogen. The same will hold for excess soil nitrogen released as a result of the "priming effect" of adding fertilizer nitrogen, to the extent to which the priming effect occurs. Hence, the effect of considering the foregoing factor would be to elevate our estimate of the contribution of fertilizer nitrogen to the nitrate of surface waters. In the interest of a conservative interpretation of our results, we chose not to introduce this consideration in our preliminary report (*2*). It should be noted also that this effect does not apply uniquely to our method, being applicable as well to the more common experiments involving isotope-enriched materials.

2) Hauck *et al.* apparently believe that variation in soil types in the Sangamon River watershed invalidates our interpretation of our data. Their contention inverts the relationship, in our report, between data and conclusion. Our data show that the value for the nitrate concentration and $\delta^{15}N$ of river samples falls on a regression line established by corresponding data from tile effluents. This line was not established, as Hauck *et al.* mistakenly assert, by two points ($\delta^{15}N = +3$ and $+13$), but rather by a least squares fit of the data for effluents from nine tiles draining an area of about 2 square miles near Cerro Gordo. Since the river samples integrate effects originating in the entire 900-square-mile watershed of the Sangamon River, we pointed out that the foregoing agreement could only be true if tile effluents represent the major source of nitrate in the entire watershed and the data from our 2-square-mile study area are representative of the mean behavior of all of the soil contained in the watershed. For example, if feedlots, which yield nitrogen at a high value for $\delta^{15}N$, contributed a significant fraction of the nitrogen found in the river samples, then the value of $\delta^{15}N$ would have been higher, for any given nitrate concentration, than the corresponding value for the tile drain effluent, since feedlots cannot possibly contribute to the latter.

Similarly, what would be the case if the soils of the intensively studied area produced nitrate with a $\delta^{15}N$ value that varied significantly from the weighted average value for the entire region? Consider the hypothetical case in which the soils of the entire region produced nitrate with a $\delta^{15}N$ value significantly lower than that for the nitrate produced by the soils of the intensively studied area. In this case, the value of $\delta^{15}N$ for any particular nitrate concentration would have fallen below the regression line. Thus, that the surface water data do not deviate significantly from the regression line establishes that the value for nitrate produced in the microregion is close to the mean value for nitrate produced throughout the drainage basin. The other possible interpretation, as we pointed out, is that a chance combination of other factors that influenced the behavior of the river caused it to duplicate the behavior of the tile effluent in the microregion.

It is possible to confront the issue of the variability of soils in the watershed directly by taking soil cores throughout the region in an attempt to average out the local variation. The enormous number of cores which would be needed to do this for a 900-square-mile area make this brute force approach impractical.

Since we had an internal control which allowed us to know that the nitrate and $\delta^{15}N$ values for the water drained from our area were close to the mean values for the entire basin, uniformity of soil type—or its absence—would have no effect on the validity of our interpretation of the river data. Nevertheless, it is perhaps worth specifying the variability of the soils of the region, since Hauck *et al.* raise the issue three times. The facts are that the soils of the Sangamon River watershed include representatives of two of the ten soil orders of the "seventh approximation" (*3*) with one of these, Mollisol, accounting for at least 80 to 90 percent of the region, excluding only the land alongside the river (*4*, figure 15). By another criterion, the soils of the watershed include 6 of the 26 soil associations described for the state of Illinois [see the general soil map of Illinois in (*4*)], with two of those associations accounting for approximately two-thirds of the total area. These are the grounds on which we based our statement that the soils of the area are "relatively uniform." We leave to the reader's taste the adequacy of our designation.

3) We have, in our report, acknowledged the fact of isotopic fractionation in the transformations of nitrogen and, of course, the nitrification of ammonia which Hauck *et al.* mention is one of these transformations. This fractionation is applicable to all ammonia, whether it is derived from soil organic matter or applied as fertilizer nitrogen. Therefore, it cannot be significant in a first-order sense. Furthermore, if this fractionation were responsible for the relationship between $\delta^{15}N$ and nitrate concentration, then the correlation between them would be positive rather than negative since the more nitrate produced the higher would be the value of $\delta^{15}N$. The more general situation is correctly stated in our report and by Hauck *et al.* In their words, "It is difficult to evaluate and make quantitative corrections for . . . isotope fractionation. . . ." However, as we noted in our report, it is not at all difficult to decide on the direction of these corrections. In this regard, we repeat the statement which we made in our report. All of the fractionations tend to understate the contribution of fertilizer nitrogen to the nitrate nitrogen appearing in surface waters. This fact makes the data interpretable where they might otherwise not be so. The only nitrogen transformation that tends to overstate the contribution of fertilizer nitrogen is nitrogen fixation, even though there is no significant fractionation accompanying it (*5*). We singled out this transformation for discussion in our report and concluded that it introduces an error about as large as that of our mass spectrometer determinations.

4) Hauck *et al.* state their belief that the mineralizable fraction of the soil is best characterized by a short-term incubation. They correctly state that our value was based on a long-term incubation (6 weeks), although their knowledge of this is based not on some guess on their part, as their words suggest, but rather on direct communication by us with Hauck and Edwards on this score. We find a compelling theoretical reason which requires the long-term in-

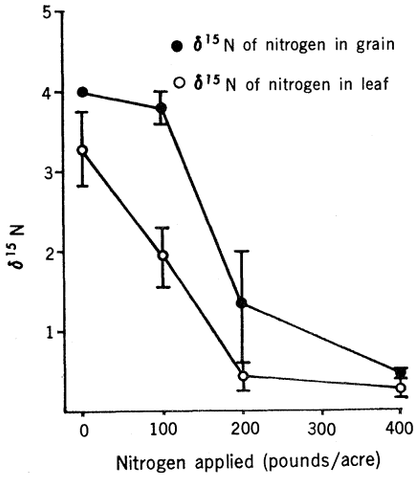

Fig. 1. Values of $\delta^{15}N$ for the total nitrogen in corn plant tissue as a function of the amount of nitrogen fertilizer applied. Urea was applied at the indicated rates (two separate plots were fertilized at each rate) just prior to planting. Each point represents the average $\delta^{15}N$ value of samples from the two plots, except for the grain sample with no fertilizer added. The extremes of the error bars indicate the average of two measurements for the sample from each of the two plots; that is, the $\delta^{15}N$ value of each sample was measured twice on the mass spectrometer and the values were averaged. On the basis of the pairs of mass spectrometer measurements, if it is assumed that (i) the machine tolerance is invariant at different $\delta^{15}N$ levels and (ii) the average of the two measurements is an unbiased estimate of the true sample value, then we can expect with 95 percent confidence that the measurement error is no greater than 0.25 $\delta^{15}N$ unit for any measurement. Leaf tissue was sampled in July 1971 and grain tissue in October 1971. The samples were supplied by L. F. Welch, Department of Agronomy, University of Illinois.

cubation. The necessary result of isotopic fractionation where ^{14}N is favored (such as during the nitrification of NH_4^+) is that the first material produced is considerably enriched in ^{14}N; that is, it has a low value of $\delta^{15}N$. If the reaction is halted before it goes to completion the $\delta^{15}N$ of the product (nitrate in this case) will necessarily be lower than the $\delta^{15}N$ of the reactant at the time that the reaction began ($t = 0$). In contrast, when the reaction is completed, the $\delta^{15}N$ of the product will be equal to the $\delta^{15}N$ of the reactant at $t = 0$.

The situation in the soil where NH_4^+ is converted to nitrate is different from the ideal case in which all of a given amount of reactant is converted to product. However, the shorter the incubation time the more will be the effect of the fractionation favoring ^{14}N, which is known to occur when NH_4^+ is converted to nitrate. The point of our experiment was to estimate the $\delta^{15}N$ value of the source (NH_4^+ derived from soil organic matter) by measuring the product (nitrate). The true value can be obtained only when the reaction has gone to completion. One will obtain an almost arbitrarily low value of $\delta^{15}N$ if the incubation is stopped soon enough. For example, in field experiments, the nitrate present in soil after the application of aqua ammonia had $\delta^{15}N$ values as low as -20, while no value even nearly that low has been observed in the drain tile effluent, the grain, or the whole plant. For these reasons the longer incubation time more nearly provides the information which is relevant (at the same time we recognize that even 6 weeks may not be long enough). If the true value is higher than the measured value, as would be the case if the incubation time were arbitrarily short, then the contribution of fertilizer nitrogen is understated.

If the soil in question has recently had fertilizer ammonia applied to it, then the longer incubation is even more important since one would anticipate an initial flush of nitrate produced from the readily available NH_4^+, a larger fraction of which may be of fertilizer rather than of soil origin. In fact this behavior has been observed (*6*). After this first flush, the rate of nitrate production decreases and reaches a steady value, which it retains for some time. It is the sum of the nitrate produced during this latter time which we take to be representative of the "mineralizable nitrogen" in the sense that the term is relevant to our experiments.

The net criticism of our work advanced by Hauck *et al.* is that environmental tracer studies based on the natural abundance ratio $^{14}N/^{15}N$ constitute "a questionable approach." Additional data will help to determine the usefulness of the approach. In Fig. 1 we offer our best example, to date, of the tracer capability of the method. Samples of grain and leaf tissue from replicate experimental plots were supplied to us by L. F. Welch of the Department of Agronomy at the University of Illinois. The corn had been grown with treatments of 0, 100, 200, or 400 pounds of nitrogen per acre; eight blind samples were supplied to us for analysis. As shown in Fig. 1, there is a systematic variation in $\delta^{15}N$ which reflects the increasing contribution of fertilizer nitrogen (low $\delta^{15}N$) relative to soil nitrogen (higher $\delta^{15}N$), notwithstanding the fact that the range of values of $\delta^{15}N$ is extremely small (about three units).

We are pleased that our work has attracted the attention of a minion of the agronomic establishment, and that it is considered significant enough to engage the efforts of the president of the Soil Science Society of America in coordinating a reply. Having dealt with the essentially technical points, we feel constrained to take note that the comments of Hauck *et al.* have a content which extends beyond purely scientific observations. First, we take exception to their serious charge regarding the "validity of the data" in the absence of any substantive evidence that our measurements of nitrate concentration and $\delta^{15}N$ are either incorrect or imprecise. We take note as well that the phrasing of their criticism gives it a grudging and occasionally insinuating tone which makes a reply more difficult than if their response were merely a "technical comment." As examples, the inverse relation of figure 1 on our report is to Hauck *et al.* an "apparent inverse relation." Our straightforward statement of the contribution of rain (based on our measurements) and estimated value of symbiotically fixed nitrogen becomes an admission. Furthermore, their comments, taken by themselves without the original report at hand, might lead the reader to believe that we have ignored the contribution of nitrogen in the rain and nitrogen fixed by soybeans and that we are unaware of the impact of isotopic fractionation on the interpretation of our results. We invite the interested reader to check our original report to assure himself that those issues are dealt with there.

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