Discussion on the Conversion Method of Soil Enzyme Stoichiometry from Different Vegetation Types in Guizhou Grassland, Southwest China

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Abstract. Soil extracellular enzymes play an important role in regulating the circulation and fixation of soil nutrients, and their stoichiometric ratio reflects the demand of microorganisms for nutrients and the supply of nutrients in the environment, so it has been widely concerned. In this study, the soil C-, N- and P-acquiring enzyme activities (β-1, 4-glucosidase, BG; β-1, 4-N-acetylglucosaminidase, NAG; leucine aminopeptidase, LAP; acid phosphatase, AP) in the upper 30 cm of soils were investigated under three vegetation types (white clover grassland, fescue grassland and natural grassland) to explore the optimal enzyme stoichiometric conversion method. The results showed that the data converted from [asin (βG / [βG + AP])] or [asin (βG / [NAG + LAP])] were more suitable to the normal distribution in general. The response of different soil enzyme conversion methods to soil depth was different. The conversion from [asin (βG / [βG + AP])] or [asin (βG / [NAG + LAP])] in 0-20 cm was more suitable to normal distribution, and the transformation of [ln (P / [1 - P])] was more suitable to normal distribution at 20-30cm. The response of different soil enzyme conversion methods to vegetation type was also different. The conversion from [asin (sqrt [βG / (βG + AP)])] or [asin (sqrt [βG / (NAG + LAP)])] was more suitable in fescue grassland, that of [ln (P / [1 - P])] was more suitable in white clover grassland, and that of [βG / AP]) or [βG / (NAG + LAP)] was more suitable in natural grassland.

1. Introduction  
The circulation of C, N, and P elements in the ecosystem is one of the hotspots in global research (Shi S et al., 2016), and the C, N, and P elements in grassland ecosystems are an indispensable and important part of the global biosphere. Extracellular enzymes produced by soil microorganisms and plant root systems promote the biogeochemical cycle of terrestrial ecosystems by decomposing and mineralizing soil organic matter. Soil extracellular enzyme activity can be a good indicator of changes in soil nutrients and substrate utilization, as well as the energy and nutrient requirements of microorganisms. Therefore, studying the activity of these enzymes can help understand the law of nutrient cycling in the ecosystem (Sinsabaugh et al., 2008; Burns et al., 2013). Many studies have
found that four soil enzymes (1, 4-β-glucosidase [βG], leucine aminopeptidase [LAP], 1, 4-β-N-acetyl glucosidase [NAG] and Phosphatase [AP]) are the main way for microorganisms to obtain C, N, and P elements (Sinsabaugh, 1994; Sinsabaugh et al., 2008). Leilei Ding, et al (2020) research found that these four extracellular enzymes (βG, NAG, LAP, AP) were identified as rigid indicators for studying the demand of soil microorganisms for C, N, and P. Enzyme stoichiometry refers to the proportion of soil extracellular enzyme activity that participates in the carbon, nitrogen, and phosphorus cycle. It reflects soil energy and nutrient limits through the metabolism and nutrient requirements of soil microorganisms and the availability of environmental nutrients (Leilei Ding, et al., 2020). Therefore, many studies have evaluated the changes in soil nutrient availability and microorganisms’ energy and nutrient requirements by analysing the activities of βG, NAG, LAP, and AP and their stoichiometry.

Soil enzyme activity and enzyme stoichiometry data are extremely important in exploring grassland ecosystems. But many studies in recent years showed that some soil enzyme stoichiometry were not suitable to the normal distribution in data statistics (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012; Moorhead et al., 2013; Burns et al., 2013). In order to better for explaining soil enzyme activity and enzyme stoichiometry, it is urgent to find a way to make the data more consistent with or close to normal distribution. There are six main research methods for transforming soil enzyme data into or near normal distribution (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012; Moorhead et al., 2013; Burns et al., 2013; Moorhead et al., 2016), i.e. 1. (βG / AP) or (βG / NAG + LAP), 2. (ln [βG] / ln [AP]) or (ln [βG] / ln [NAG + LAP]), 3. (βG / βG + AP) or (βG / NAG + LAP), 4. asin (βG / [βG + AP]) or asin (βG / [NAG + LAP]), 5. asin (sqrt [βG / (βG + AP)]) or asin (sqrt [βG / (NAG + LAP)]), 6. ln (P / [1 - P]), where P = βG / (βG + AP) or βG / (βG + NAG + LAP). Based on these, we use the above 6 analysis methods to compare and analyse that which method can better for analysing the soil enzyme stoichiometry under different vegetation types in Guizhou grassland. Regional grassland soil enzyme stoichiometry analysis provides theoretical support.

2. Materials and methods

2.1. Overview of the plot
The test area is in Weining County, northwest of Guizhou Province. It has a subtropical monsoon humid climate, with 1812 hours of sunshine per year, 180 days of frost-free period, annual rainfall of 926 mm, small annual temperature difference, large daily temperature difference, warm winter and cool summer, and summer. The average temperature is 18 degrees. The test plot is in the Guiran Demonstration Farm of Weining Plateau Experimental Station in Guizhou Province. The ranch was built in 1986 and covers an area of 470 hectares, which can provide good test conditions for the test.

2.2. Experimental design
Three typical grasslands (white clover grassland, fescue grassland, and natural grassland) in Guizhou Plateau were selected. Each grassland area was 100m×100m. Six grassland plots were set along the S shape in each grass to repeat. 2m×2m, each square is 30m apart. In the set sample, use a soil drill to collect samples in layers of 0-10cm, 10-20cm, and 20-30cm. Repeat the sampling three times for each soil depth. After thoroughly mixing the soil samples obtained from each layer, take samples and obtain the final samples. The sealed soil sample is taken back to the laboratory as a soil sample.

Determination of β-1,4-glucosidase (βG), soil leucine aminopeptidase (LAP), soil acid phosphatase (AP) and β-N-acetylamino grape in soil Nuclease (NAG) by kit double antibody sandwich method (Leilei Ding, et al, 2020).
2.3. Data analysis methods

Use Microsoft Excel (Office for Mac 2016) to calculate the kurtosis and skewness of the normal distribution of all data, perform a normal test on the calculated data, and find a conversion method for the kurtosis and skewness that conform to the normal distribution.

3. Results and analysis

3.1. Normal analysis of grassland soil enzyme stoichiometric ratio

As can be seen from Table 1, βG / AP, ln (βG) / ln (AP), βG / (βG + AP), asin [sqrt (βG / [βG + AP])], and ln (P / [1 - P]) converted data do not conform to the normal distribution, while asin (βG / [βG + AP]) converted data are closer to the normal distribution; βG / (NAG + in the enzyme C: N ratio LAP), ln (βG) / ln (NAG + LAP), βG / (βG + NAG + LAP), asin (sqrt [βG / (βG + NAG + LAP)]), and ln (P / [1 - P]) converted data are closer to the normal distribution. Therefore, an overall analysis of the data reveals that [asin (βG / [βG + AP])] or [asin (βG / [βG + NAG + LAP])]) has more kurtosis and skewness than the other methods meet the normal distribution.

| Table 1. Kurtosis and skewness of the six conversion methods in the overall analysis. |
|---------------------------------|---------------------------------|
| variable                        | βG vs AP                        | βG vs (NAG + LAP)                       |
| Ratio                           | Mean ± Std Dev                  | Kurtosis                                |
| Ratios                          | 1.73±0.43                       | 0.49                                    |
| Proportions                     | 0.63±0.06                       | -0.65                                   |
| Ln (ratios)                     | 0.42±0.21                       | 0.45                                    |
| Asin (proportions)              | 0.68±0.07                       | 0.02                                    |
| Asin (sqrt [proportions])       | 0.91±0.06                       | 0.08                                    |
| Logit (proportions)             | 0.52±0.25                       | 0.12                                    |

3.2. Normal analysis of soil enzyme stoichiometric ratio at different soil depths

It can be obtained from Table 2 that when the soil depth is 0-10cm and 10-20cm, βG / AP, ln (βG) / ln (AP), βG / (βG + AP), asin [ The data transformed by sqrt (βG / [βG + AP])] and ln (P / [1 - P]) do not conform to the normal distribution, while the data transformed by asin (βG / [βG + AP]) are closer to normal distribution; βG / (NAG + LAP), ln (βG) / ln (NAG + LAP), βG / (βG + NAG + LAP), asin (sqrt [βG / (βG + NAG + LAP)]), and ln (P / [1 - P]) converted data are closer to the normal distribution. Therefore, an overall analysis of the data reveals that [asin (βG / [βG + AP])] or [asin (βG / [βG + NAG + LAP])]) has more kurtosis and skewness than the other methods meet the normal distribution.

| Table 2. Kurtosis and skewness of the six conversion methods at different soil depths. |
|---------------------------------|---------------------------------|
| variable                        | βG vs AP                        | βG vs (NAG + LAP)                       |
| Ratio                           | Mean ± Std Dev                  | Kurtosis                                |
| Ratios                          | 1.73±0.43                       | 0.49                                    |
| Proportions                     | 0.63±0.06                       | -0.65                                   |
| Ln (ratios)                     | 0.42±0.21                       | 0.45                                    |
| Asin (proportions)              | 0.68±0.07                       | 0.02                                    |
| Asin (sqrt [proportions])       | 0.91±0.06                       | 0.08                                    |
| Logit (proportions)             | 0.52±0.25                       | 0.12                                    |

In conclusion, at different soil depths, there are different conversion methods that conform to the normal distribution of soil enzyme metering ratio data. When the soil depth is 0-20cm, ([asin (βG / [βG + AP])] or [asin (βG / [βG + NAG + LAP])]) is more kurtosis and skewness than the others This method is more consistent with the normal distribution; when the soil depth is 20-30cm, the kurtosis and skewness of the data obtained by the ln (P / [1 - P]) conversion are more consistent with the normal distribution than the other methods.
Table 2. Kurtosis and skewness of 6 conversion methods at different soil depths.

| Different soil depth | variable                  | βG vs AP               | βG vs (NAG + LAP)          |
|----------------------|---------------------------|------------------------|---------------------------|
|                      |                           | Mean ± Std Dev Kurtosis| Mean ± Std Dev Kurtosis   |
|                      | Ratios                    | 1.71±0.41              | 0.50±0.09                |
| 0-10cm               | Proportions               | 0.62±0.06              | 0.33±0.04                |
|                      | Ln (ratios)               | 0.34±0.21              | 0.34±0.04                |
|                      | Asin (proportions)        | 0.68±0.07              | 0.34±0.04                |
|                      | Asin (sqrt [proportions])| 0.91±0.06              | 0.61±0.41                |
|                      | Logit (proportions)       | 0.51±0.24              | -0.71±0.18               |
|                      | Ratios                    | 1.63±0.35              | 0.48±0.08                |
|                      | Proportions               | 0.61±0.50              | 0.32±0.04                |
| 10-20cm              | Ln (ratios)               | 0.46±0.19              | -1.15±0.79               |
|                      | Asin (proportions)        | 0.66±0.06              | 0.33±0.04                |
|                      | Asin (sqrt [proportions])| 0.90±0.05              | 0.60±0.40                |
|                      | Logit (proportions)       | 0.47±0.21              | -0.76±0.17               |
|                      | Ratios                    | 1.85±0.51              | 0.51±0.12                |
|                      | Proportions               | 0.64±0.06              | 0.34±0.05                |
| 20-30cm              | Ln (ratios)               | 0.46±0.20              | -0.79±5.41               |
|                      | Asin (proportions)        | 0.69±0.83              | 0.34±0.05                |
|                      | Asin (sqrt [proportions])| 0.93±0.67              | 0.62±0.05                |
|                      | Logit (proportions)       | 0.58±0.28              | -0.69±0.22               |

3.3. Normal analysis of soil enzyme stoichiometric ratio under different vegetation types

According to Table 3, βG / AP, ln (βG) / ln (AP), βG / (βG + AP), asin (βG / [(βG + AP)]) and The data transformed by ln (P / [1 - P]) do not conform to the normal distribution, while the data transformed by asin [sqrt (βG / (βG + AP))] are closer to the normal distribution; βG in the enzyme C: N ratio / (NAG + LAP), ln (βG) / ln (NAG + LAP), βG / (βG + NAG + LAP), asin (βG / [βG + NAG + LAP]), and ln (P / [1 - P]) The transformed data do not conform to the normal distribution, while the asin (sqrt [βG / (βG + NAG + LAP)]) transformed data are closer to the normal distribution. B Г / AP, ln (βG) / ln (AP), βG / (βG + AP), asin (βG / [(βG + AP)], and asin [sqrt (βG / [ βG + AP])] converted data do not conform to the normal distribution, while ln (P / [1 - P]) converted data are closer to the normal distribution; βG / (NAG + LAP), ln (βG) / ln (NAG + LAP), βG / (βG + NAG + LAP), asin (βG / [βG + NAG + LAP]), and asin (sqrt [βG / (βG + NAG + LAP)]) the transformed data do not conform to the normal distribution, while the data transformed by ln (P / [1 - P]) is closer to the normal distribution. Ln (βG) / ln (AP), βG / (βG + AP), asin (βG / [(βG + AP)], asin [sqrt (βG / [ βG + AP])] And ln (P / [1 - P]) conversion data do not conform to the normal distribution, while βG / AP conversion data are closer to the normal distribution; ln (βG) / ln (AP) in the enzyme C: N ratio , βG / (βG + AP), asin (βG / [(βG + AP)], asin [sqrt (βG / [ βG + AP])] and ln (P / [1 - P]) conversion data do not match Normal distribution, while βG / AP conversion data is closer to normal distribution.

In summary, under the treatment of 6 different conversion methods, the conversion methods that conform to the normal distribution of soil enzyme data of different vegetation types are different. Soil enzyme metering ratio of fescue grassland ([asin (sqrt [βG / (βG + AP)]) or [asin (sqrt [βG / (NAG + LAP)])]) conversion of the kurtosis and skewness ratio of the data Several other methods are more in line with the normal distribution and are more suitable for calculating soil enzyme metering ratios of Festuca arundinacea; while soil enzyme metering ratios of white clover grassland [ln (P / [1 - P])] transform the kurtosis and skewness is more consistent with the normal distribution than several other methods, and is more suitable for calculating soil enzyme metering ratio of white clover grassland; natural grassland enzyme metering ratio ([βG / AP]) or ([βG / (NAG + LAP)]) conversion the kurtosis and skewness of the obtained data are more consistent with the normal distribution than several other methods.
Table 3. Kurtosis and skewness of 6 conversion methods under different vegetation types.

| Different vegetation types | variable                      | βG vs AP | Mean ± Std Dev | Skew  | Kurtosis |
|----------------------------|-------------------------------|----------|----------------|-------|----------|
|                            |                               | βG vs (NAG + LAP) | Mean ± Std Dev | Skew  | Kurtosis |
| Festuca                    | Ratios                        | 1.62±0.47 | 0.81 | -0.30 | 0.49±0.09 | 0.32 | -0.85 |
|                            |                              | 0.61±0.06 | 0.37 | -1.13 | 0.33±0.04 | 0.13 | -1.00 |
|                            | Ln (ratios)                   | 0.51±0.23 | 0.20 | -1.22 | -0.90±2.85 | 3.00 | 11.73 |
|                            | Asin (ratios)                 | 0.65±0.08 | 0.45 | -1.03 | 0.33±0.04 | 0.16 | -0.98 |
|                            | Asin (sqrt [ratios])          | 0.89±0.07 | 0.11 | -0.27 | 0.61±0.04 | 0.08 | -1.01 |
|                            | Logit (ratios)                | 0.44±0.28 | 0.45 | -1.01 | -0.73±0.19 | 0.37 | -1.02 |
|                            | Ratios                        | 1.67±0.40 | 0.82 | -0.54 | 0.50±0.11 | 0.98 | 0.71 |
|                            |                              | 0.62±0.05 | 0.52 | -1.13 | 0.33±0.05 | 0.65 | 0.80 |
| Clover                     | Ratios                        | 0.45±0.18 | -0.44 | -0.55 | -0.77±4.66 | 2.85 | 11.61 |
|                            |                              | 0.67±0.07 | 0.57 | -1.04 | 0.34±0.05 | 0.69 | 0.15 |
|                            | Asin (ratios)                 | 0.91±0.05 | 0.55 | -1.08 | 0.61±0.05 | 0.57 | -0.01 |
|                            | Asin (sqrt [ratios])          | 0.91±0.23 | 0.38 | -1.03 | 0.71±0.22 | 0.50 | -0.08 |
|                            | Logit (ratios)                | 0.65±0.05 | -0.30 | -0.07 | 0.33±0.04 | -0.40 | 0.11 |
|                            | Ratios                        | 1.91±0.40 | 0.37 | 0.02 | 0.50±0.08 | -0.11 | -1.14 |
|                            |                              | 0.30±0.16 | 1.39 | 1.30 | -0.76±0.66 | -1.66 | 2.05 |
| Nature                     | Ratios                        | 0.71±0.61 | -0.17 | -0.14 | 0.34±0.40 | -0.36 | 0.07 |
|                            |                              | 0.94±0.05 | -0.22 | -0.10 | 0.61±0.04 | -0.48 | 0.21 |
|                            | Asin (ratios)                 | 0.62±0.21 | -0.15 | -0.12 | -0.72±0.17 | -0.56 | 0.33 |

4. Conclusion
In this study, the soil C-, N- and P-acquiring enzyme activities (β-1, 4-glucosidase, BG; β-1, 4-N-acetylglucosaminidase, NAG; leucine aminopeptidase, LAP; acid phosphatase, AP) in the upper 30 cm of soils were investigated under three vegetation types (white clover grassland, fescue grassland and natural grassland) to explore the optimal enzyme stoichiometric conversion method. The results showed that:

1. On the whole, data converted from [asin (βG / βG + AP)] or [asin (βG / (NAG + LAP))] were more suitable to the normal distribution in general.

2. The response of different soil enzyme conversion methods to soil depth was different. The conversion from [asin (βG / βG + AP)] or [asin (βG / (NAG + LAP))] in 0-20 cm was more suitable to normal distribution, and the transformation of [ln (P / [1 - P])] was more suitable to normal distribution at 20-30 cm.

3. The response of different soil enzyme conversion methods to vegetation type was also different. The conversion from [asin (sqrt (βG / (βG + AP)))] or [asin (sqrt (βG / (NAG + LAP)))] was more suitable in fescue grassland, that of [ln (P / [1 - P])] was more suitable in white clover grassland, and that of [βG / AP] or [βG / (NAG + LAP)] was more suitable in natural grassland.

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References
[1] Shi, S., Peng, C., Wang, M., Zhu, Q., Yang, G., & Yang, Y., et al. A global meta-analysis of changes in soil carbon, nitrogen, phosphorus and sulfur, and stoichiometric shifts after forestation. Plant and Soil, 407 (1-2) (2016) 323-340.
[2] Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., & Zeglin, L. H. Stoichiometry of soil enzyme activity at global scale. Ecology Letters, 11 (11) (2008) 1252-1264.
[3] Burns, R. G., Deforest, J. L., Marxsen, Jürgen, Sinsabaugh, R. L., Stromberger, M. E., & Wallenstein, M. D., et al. Soil enzymes in a changing environment: current knowledge and...
future directions. Soil Biology and Biochemistry, 58 (2013) 216-234.

[4] Moorhead, D. L., Sinsabaugh, R. L., Hill, B. H., & Weintraub, M. N. Vector analysis of ecoenzyme activities reveal constraints on coupled c, n and p dynamics. Soil Biology and Biochemistry, 93 (2016) 1-7.

[5] Sinsabaugh, R. L., & Follstad Shah, J. J. Ecoenzymatic stoichiometry and ecological theory. Annual Review of Ecology, Evolution, and Systematics, 43 (1) (2012) 313-343.

[6] Sinsabaugh, R. S. Enzymic analysis of microbial pattern and process. Biology and Fertility of Soils, 17 (1) (1994) 69-74.

[7] Moorhead, D. L., Rinkes, Z. L., Sinsabaugh, R. L., & Weintraub, M. N. Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. Frontiers in Microbiology, 4 (223) (2013) 1-12.

[8] Leilei Ding, Yishun Shang, Wen Zhang, Yu Zhang, Shige Li, Xin Wei, Yujun Zhang, Xuelian Song, Xi Chen, Jiayia Liu, Fuli Yang, Xuedong Yang, Chao Zou, Puchang Wang. Disentangling the effects of driving forces on soil bacterial and fungal communities under shrub encroachment on the Guizhou Plateau of China. Science of the Total Environment, 1 (25) (2020) 709.