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Sulphur and nitrogen content as sulphur deficiency indicator for grasses

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ABSTRACT

In Europe, during the last 30 years, the decrease in sulphur (S) atmospheric depositions has led to S deficient grasslands. Concern for S fertilisation resulted in research about S fertilisation advices and definition of S nutritional diagnostic tools for plants. However, for grasses, S nutrition indicators are still discussed. We propose a diagnostic tool based upon linear relationships linking the sulphur and nitrogen (N) content of grasses. This diagnostic tool is built thanks to data from field and pot trials treated with an algorithm (Bolides) and discriminant analyses. The relationships allow the characterisation of the grass sulphur nutritional status following four categories: certainly sufficient, probably sufficient, probably deficient and certainly deficient. This relation based and tested on a large dataset from literature and own field trials allowed diagnosing correctly 94% of the sulphur sufficient grasses and 71% of the sulphur deficient grasses.

1. Introduction

During the last few decades, a decrease in sulphur (S) deposits (McGrath et al., 2002) and lower S supply through mineral fertilisation (Ceccotti, 1996) has led to sulphur deficient crops and cutting grasslands throughout Europe (Zhao et al., 2002). A wide range of yield responses to sulphur fertilisation has been observed for grasslands, from 0.24 (Stevens and Watson, 1986) to 3.5 t DM ha^{-1} y^{-1} (Brown et al., 2000). Yield increases with S fertilisation were also observed in some of the highest S deposition areas in Europe (Tarrason et al., 2003) such as in Belgium (Mathot et al., 2008) and in the Netherlands (Bussink and Den Boer, 2000).

Some authors (Scott, 1981; Zhao and McGrath, 1994) proposed soil S analysis as an indicator for S availability for plant growth. However, considering the high mobility of S in soil and the effects of many other factors such as the climate variation and the deepness of the water table, soil analysis is sometimes inadequate for predicting S deficiencies at the parcel scale (Verlinden, 2002). Balance (in-out) at the parcel level can also be used as an indicator of plant available S (Schnug and Haneklaus, 1998). However, at the field scale basis, for practical fertilisation decisions, a lot of information is required with an accuracy not always available (Oenema and Postma, 2003). Plant analyses have been frequently quoted as useful for diagnosing the S nutrition of crops (Black Kalff et al., 2002). Using a good S deficiency indicator based on grass analyses can be a very complementary tool for methods predicting S deficiency. It can allow to have information on the real S nutrition of the plants and therefore to evaluate the predicting methods without having to set up experimental trials with several S treatments and yield measurements.

Many parameters have been proposed as plant S deficiency indicators (reviewed among others by Dijkshoorn and Van Wijk, 1967; Schnug and Haneklaus, 1998; Black Kalff et al., 2002) such as total S, sulphate, organic S or ratios as, nitrogen (N):S, malate:sulphate, sulphate:total S; but, till date, no indicator has been chosen for grasses. The most commonly used indicators are S content and N:S ratio. However, literature reports that grasses with S content from 0.95 mg S g^{-1} DM (Helgadottir et al., 1977) to 2.5 mg S g^{-1} DM (Eppendorfer, 1976) or N:S ratio from 14 (Bolton et al., 1976) to 20 (Eppendorfer, 1976) responded positively to S fertilisation. Discussions on these indicators focus on critical values variation not only with nitrogen nutrition of the plants (Kowalenko, 2004) but also during plant growth. Indeed, as for nitrogen (Lemaire and Salette, 1984), phosphorus (P) and potassium (K) (Salette, 1982), S content and therefore the critical values decrease during plant growth (Salette, 1978). According to Duru and Thélier Huché (1997), K and P nutrition status of grasses can be estimated by comparing K or P grass content to Index values taking into account a linear relationship between these elements and the grass N content. Considering that S content also decrease during plant growth (Salette, 1978) and that, such as for K, luxurious consumption of S occurs, it is proposed to define an indicator method for diagnosing sulphur nutrition of grasses based on their S and N content.

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Table 1
Data source, type of experiment and origin, number of site, sward species and number of data utilised from each source in the sulphur deficient (def) or sufficient (suf) database.

Source	Type of experiment and origin[a]	Number of site	Sward species	Number of data	
				Def	Suf
Bolton et al., 1976	Pot experiment	/	Lp[1]	7	38
Brown et al., 2000	Field (UK)	2	Lp	2	9
Eppendorfer, 1976	Pot experiment	/	Lm[2]	7	80
Hahtonen and Saarela, 1995	Field (Fin)	6[a]	Pp[3], Dg[4] and Fp[5]	0	80
Kowalenko, 2004	Field (Can)	1[b]	Dg, Lp, Fa[6], Tp[7] and Tr[8]	3	15
Mathot et al., 2005	Pot experiment	/	Lp	12	79
Mathot et al., 2008	Field (Bel)	8	Lp and Lm	14	366
Mertens, 2005	Field (Bel)	2	Lp	2	49
Morris et al., 1994	Field (USA)	1	Lm	0	54
Murphy et al., 2002	Field (Irl)	1[b]	Lp and Tr	2	4
Stevens and Watson, 1986	Field (N-Irl	20[c]	n.c.	10	42
Total				59	816

[1] Lolium pratense.
[2] Lolium multiflorum.
[3] Phleum pratense.
[4] Dactylis glomerata.
[5] Festuca pratensis.
[6] Festuca arundinacea.
[7] Trifolium pratense.
[8] Trifolium repens.
[a] Each site is a pure stand of Pp, Dg or Fp.
[b] Less than 20% of Tp or Tr.
[c] Reported as grass for silage.

2. Materials and methods

2.1. Data collection

Data were collected from literature or own field and pot experiments (Table 1). The data collected were included in the database whatever the grass species. However, no data from swards containing more than 20% of legumes were taken into account (Duru and Thélier Huché, 1997). All the data are grass analysis related to S fertilisation experiments. For each data the N and S contents are the mean of 4 replicates of grass analysis of a given treatment of one cut (no annual averages). The indicators proposed hereafter are calculated using only N and S content of grasses.

No yield values are necessary. Field and pot trials data are mixed together without distinction because of the good repartition of those two kinds of data (Fig. 1).

For field trials data, Salette and Thélier (1991) restricted the validity domain of the nutritional Index value and the mineral dilution curve to grasses with a dry matter production ranging from 2 to 5 t DM ha^{-1} and with a N content ranging from 15 to 45 mg N g^{-1} DM. Because of similarity, we excluded from the database the data out of the ranges mentioned by these authors.

Data were sorted in two different pools, deficient or sufficient according to the author's observations (Table 1). Deficient grasses were swards that responded significantly (dry matter yield increases) to sulphur fertilisation as observed in the experiments. Sulphur sufficient grasses are all the other data (no effect of S fertilisation on the yield).

2.2. Indicator definition

Considering the repartition of the data (Fig. 2), three zones can be intuitively distinguished. Zone 1, for the grasses with the highest S content, corresponds mainly to S sufficient grasses. Zone 2, an incertitude zone, includes both S deficient and sufficient grasses. Finally, zone 3, for the lowest S content, includes mainly S deficient grasses. The limits of the three zones will be defined thanks

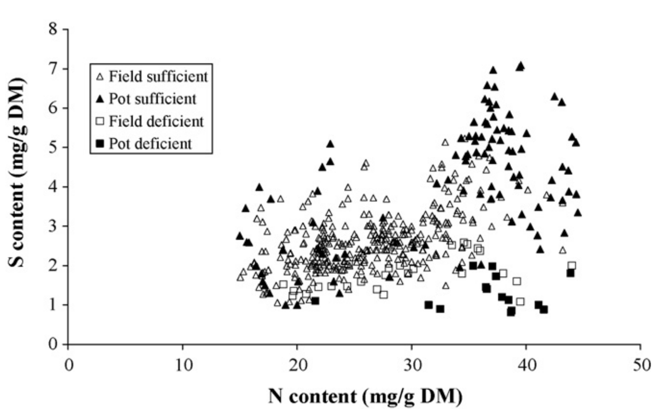

Fig. 1. Repartition of the data in function of the experimental procedure (pot or field).

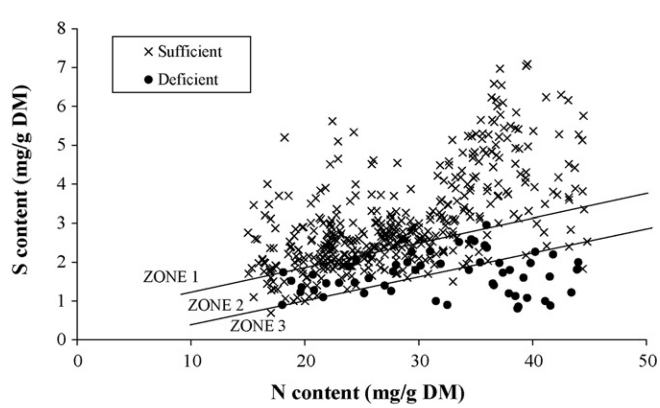

Fig. 2. Sulphur content in function of N content of the grasses of sulphur deficient and sufficient grasses. Overview of the three main zones of sulphur nutritional status. In zone 1, grasses are mainly not S deficient, zone 2 is an incertitude area and zone 3 contains mainly S deficient grasses.

to an algorithm (Bolides) and a statistical procedure (discriminant analysis).

The zones 1 and 3 can be delimited thanks to an algorithm called Bolides, fully described by Schnug et al. (1996). This method "enables a mathematically correct and reproducible fitting of boundary lines on individual classes of XY scattered data". This procedure includes as a first step the removal of outlier data *i.e.* data that are too separated from other data in terms of nutrients content. For that, three parameters: n, σx and σy are used. Considering a scattered plot with N content as the X axis and S content as the Y axis, $n-1$ other data than the one tested have to be present in a rectangle centred on the data tested. The size of this rectangle is defined by σx and σy which are respectively the length of the side of the rectangle on the X (N content) and Y axis (S content). In this study the parameters used were $n=2$ and σx and σy the mean N and S content , respectively, of the dataset multiplied by the standard deviations observed by Crosland et al. (2001) for the chemical determinations of nitrogen (0.036) and total sulphur (0.16) in plants. On a second step, the boundary line is drawn for the sufficient and deficient set of data thanks to the mathematical procedure described by Schnug et al. (1996). Because of similarity to the relation between N and P or K reported by Duru and Thélier Huché (1997) the boundary lines were drawn as linear regressions. However, these linear regressions were calculated from only a selected zone of the datasets. Indeed, in the extreme N content, the density of data is far less than that in the middle range. For the definition of the S sufficient grass boundary lines, we excluded the boundary points calculated from the data with higher S content but lower N content (left of the point a in Fig. 3) than the data with the lowest S content (point a in Fig. 3). For the definition of the boundary line of the S deficient data, we excluded the boundary points calculated from the data with a lower S content but a higher N content (right of point b in Fig. 4) than the data with the highest S content (point b). This selection can be justified as follows: the boundary line is defined using limit points that are supposed to be barely sufficient or deficient. However, for example, for the definition of the boundary line of the sulphur deficient data (Fig. 4), average yield decreases for grasses with N content lower than the grass with the highest S content is on average of 15%. At the opposite, the average yield decrease for the grass with N content higher than the N content of the data with the highest S content is of about 45%. This indicates that those data are strongly deficient and they should not be used for the boundary line definition.

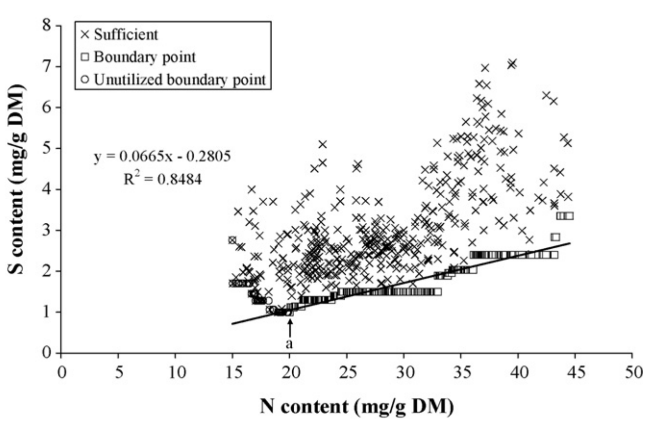

Fig. 3. Boundary line 1 definition for the S sufficient data. Data were selected for outliers ($n=2$; $\sigma x=1.09$ and $\sigma y=0.39$) and regression calculated on the boundary points with N content higher than the N contents of the grass with the lowest S content.

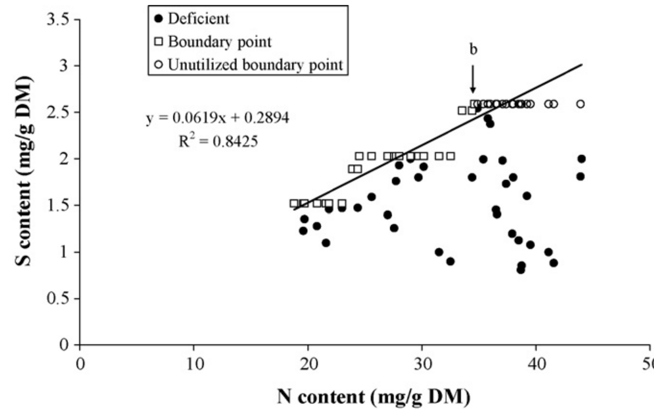

Fig. 4. Boundary line 2 definition for the S deficient data. Data were selected for outliers ($n=2$; $\sigma x=1.25$ and $\sigma y=0.22$) and regression calculated on the boundary points with N content lower than the N contents of the grass with the highest S content.

We delimited the *first zone* (sufficient grasses) downwards by the boundary line 2 (Figs. 4 and 6) calculated with the deficient dataset. In principle, for a given N content all the grasses having a S content higher than the one calculated with this boundary line 2 have an adequate sulphur nutritional status. The *third zone* (deficient data) was delimited upwards by the boundary line 1 (Figs. 3 and 6) calculated with the S sufficient dataset. Similarly, in principle, for a given N content all the grasses having a S content lower than the one calculated with this boundary line 2 have an inadequate sulphur nutritional status for optimal plant growth.

Ideally the two boundary lines should fit each other. However, as showed hereafter (Fig. 5) there is a gap between the two lines, corresponding to the incertitude zone (zone 2) where sufficient and deficient data are mixed. For the data included within that zone, a discriminant analysis procedure was used to calculate the linear relation between N and S content delimiting the two sets of data with the highest probability of well diagnosing the sulphur nutrition of the grasses (Dagnelie, 1975; Systat, 1998). Nitrogen and sulphur contents of grasses were the variables and the nutritional status (deficient or sufficient) were the 2 populations.

3. Results

The removal of outliers and selection on range of N content and DM yield led to an important reduction of the database. There were respectively 430 and 42 data left in the sufficient and deficient databases.

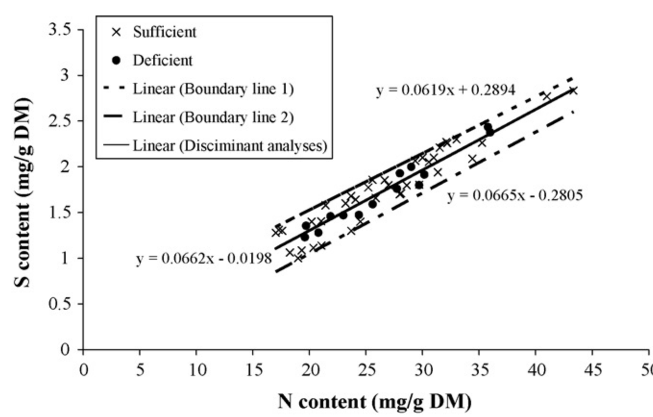

Fig. 5. Discriminant curve definition in the uncertainty zone.

Table 2
Critical linear relationships for diagnosing sulphur nutritional status of grasses.

Zones	Diagnostic	Critical relationships	Equation determination
1. Sufficiency	S > Ss, certainly sufficient	Ss = 0.0619 × N + 0.2894	Boundary line 2
2. Uncertainty	Ss > S > Su, probably sufficient	Su = 0.0662 × N−0.0198	Discriminant analysis
	Su > S > Sd, probably deficient		
3. Deficiency	Sd > S, Certainly deficient	Sd = 0.0665 × N−0.2805	Boundary line 1

Table 3
Repartition (%) of the data in function of their real sulphur nutritional status within the three zones.

Real nutritional status	Diagnostic zones			
	Zone 1 (sufficiency) S > Ss	Zone 2 (uncertainty) Ss > S > Su	Su > S > Sd	Zone 3 (deficiency) Sd > S
Sufficient (n = 430)	89	5	4	2
Deficient (n = 42)	14	14	19	52

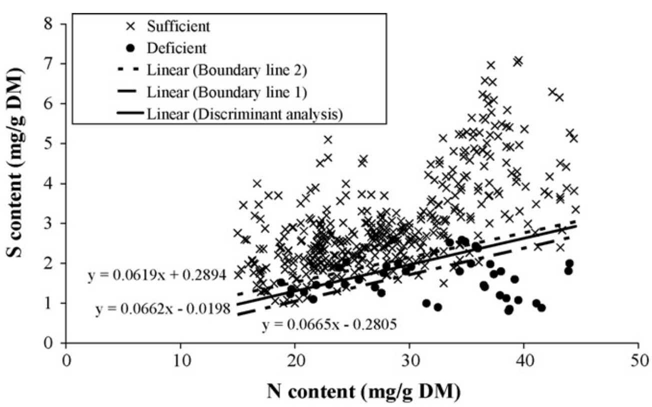

Fig. 6. Boundary line 1, 2 and the discriminant linear relation for delimiting the sulphur nutritional status zones.

The boundary lines 1 and 2 were calculated (Figs. 3 and 4). Between the boundary lines 1 and 2 is the second zone (incertitude) with respectively 14 and 38 deficient and sufficient grasses. Within this zone the linear regressions calculated with the discriminant analysis (Fig. 5) led to a correct sulphur nutrition diagnosing of 58% of the data (58% for the sufficient and 57% for the deficient data).

The procedure reported above resulted in the definition of three linear relationships between sulphur and nitrogen allowing the characterisation of grass sulphur nutritional status (Fig. 6 and Table 2).

Grass with an S content higher than Ss can be considered as certainly sufficient. Forage having an S content between Ss and Su is probably sufficient. While the S content of the grass is lower than Su but higher than Sd the forage is probably deficient and, finally, with an S content lower than Sd the forage is considered as certainly sulphur deficient.

4. Discussion

All the data were put into the database for the determination of the boundary lines because too low S deficient grasses were available to divide each dataset (mainly the deficient) to create a representative dataset for validating the indicator. Therefore, independent probabilities of well diagnosing the status of grass are not available. Anyway, the repartition of the data in function of their sulphur nutritional status in the zones is showed in Table 3. For the sulphur sufficient data, 94% are diagnosed correctly with a supposed high certainty for 95% of them. For the sulphur deficient grasses, 71% are diagnosed correctly with a supposed high certainty for 73% of them.

Considering the utilisation of boundary line for the definition of the sufficiency and deficiency critical curve, error risk is certainly very low in the corresponding zones. In the incertitude zone, the discriminant analysis method was used to maximize the probability of well diagnosing sulphur status of grass. This probability is still quite low, close to 60%, indicating real difficulties of diagnosing S nutritional status in that zone. For grasses included in that zone, it could be useful to consider other agricultural information such as soil type, climate, atmospheric S deposition,. . . to diagnose more precisely the S nutrition of the grasses. Despite this low probability, it should be noticed that the incertitude zone is quite narrow, on average 0.43 mg/g DM of S, in the range content from 15 to 45 mg/g DM of nitrogen content. It would however be interesting to validate this method with a large independent set of data. Taking into account the multiple analytical methods used for the grass sulphur content determination used as data and the potential variability (variation coefficient of 16%) inherent in the use of different methods reported by Crosland et al. (2001), the diagnostic tool based on the 3 critical curves seems to be accurate.

More generally, as for K and P (Duru and Thélier Huché, 1997), the determination of S nutritional status using the relationships between sulphur and nitrogen content of the grasses includes the effects of the N nutrition and the dilution of the grass S content during growth. It does not require any accurate yield measurement, only an estimation to fit the validity range. This diagnostic tool is therefore also suitable for large survey out of experimental fields. As recommended by Salette and Thélier (1991) this validity ranges should however be respected because of, on one hand, at low yield and high N content, the evolution of the nitrogen and mineral content of the grasses with growth may differ mainly at the beginning of the growth, and on the other hand, at high yield and low N content, the physiological changes (lignification of the stems, scenecence of leaves, . . .) may modify the repartition of the elements within the plants. Practically, the validity range proposed corresponds to cutting grassland yields for silage in commercial farms and therefore could be easily used for routine analysis. However, because the sulphur requirements and content of legumes may differ from grasses in a mixed sward (Metson and Saunder, 1978) attention should also be given as to not have a too high proportion of it in the sward. A proportion of less than 20% of legumes seems to be suitable. For large survey fast methods such as NIRS could be used for the determination of legume proportion in the cover (Deprez et al., 2005) to ensure to be in the utilisation conditions of the diagnostic tool. Furthermore, as for P and K (Jouany et al., 2005), correction coefficient for sulphur deficiency indicator for grassland in presence of legumes should be defined.

In the future, using the simple diagnostic tool proposed here above could be useful for developing prediction methods for estimating the sulphur supply to grass but also to make S fertilisation advices. Sulphur fertilisation could be considered while S nutritional value indicates shortage in S supply for the sward. Attention should be given to the influence of factors such as the nitrogen nutrition of the grasses or climatic conditions. The sulphur nutritional indicator gives an *a postiori* information on the S nutrition of the sward and therefore, changes in fertilisation practices (mainly N) or growing conditions may lead to change in S requirement of the sward. However, as the management of permanent grassland is generally quite similar year after year in commercial farms, the diagnosis of sulphur nutrition could be useful to drive the fertilisation.

5. Conclusion

We propose three linear relationships linking sulphur and nitrogen content of grasses as sulphur nutritional diagnostic tool. Thanks to these three linear relationships, the S status of plants can be characterised into the following classes: certainly sufficient, probably sufficient, probably deficient and certainly deficient. This method should ideally be validated on an independent large set of data and should be used for grasses with nitrogen content and yield ranging respectively from 15 to 45 mg N g^{-1} DM and 2 to 5 t DM ha^{-1}. In the future, this diagnostic tool requiring only grass S and N content analysis could be very useful for evaluating the sulphur nutritional status of numerous grasslands.

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