MULTIPLEX : A NEW DIAGNOSTIC TOOL FOR MANAGEMENT OF NITROGEN FERTILIZATION OF TURFGRASS

S. Lejealle, S. Evain

FORCE-A
Orsay, France

Z. G. Cerovic

ESE-CNRS laboratory
Orsay, France

ABSTRACT

Multiplex is a fluorescence-based optical sensor that measures in real time and in vivo the leaf content of compounds such as chlorophyll and several families of polyphenols (anthocyanins, flavonoïds, hydroxycinnamic acids). We propose here to show that the measurement of leaf chlorophyll and flavonoïds content permits us to evaluate nitrogen status of turfgrass. This study has shown that chlorophyll content increases whereas flavonoïds content decreases with increased nitrogen application. So, the chlorophyll-to-flavonoïds ratio named nitrogen balance index (NBI) increases with nitrogen quantity applied on turfgrass. Moreover, NBI measured by Multiplex is well correlated with turfgrass leaf-nitrogen content, better than chlorophyll content alone. Thanks to the NBI, Multiplex measurements permit us to evaluate without any sample preparation quickly and in real time turfgrass nitrogen status. Also, Multiplex gives an objective reference of turfgrass nitrogen level and can help nitrogen fertilization management. By following NBI kinetics of turfgrass, we can see the variation of nitrogen status and particularly when leaf-nitrogen content decreases. This decrease indicates that the fertilizer has been totally used up by turfgrass and that new fertilization is needed. This could avoid low or over-fertilization that is known to increase turfgrass sensitivity to diseases. We have shown also that Multiplex measurements permits mapping on NBI on sport fields, therefore, nitrogen fertilization could be adapted spatially and in a timely manner to respond to turfgrass needs.

Keywords: turfgrass, nitrogen status, fertilization, diagnostic tool, mapping.

INTRODUCTION

Nitrogen is a really important nutrient in turfgrass nutrition to keep enough density, a good color and to allow turfgrass to regenerate after many stresses like wear, aerations or diseases. Nitrogen is responsible of turfgrass vitality and resistance to diseases pressure, but over-fertilization can increase susceptibility to several fungal diseases like Michrodochium nivale and pythiums (Beard, 2002). Nitrogen management depends on each turfgrass use. For soccer or rugby playgrounds, a high level of nitrogen is applied to maintain turfgrass growth to regenerate after matches whereas on golf courses a low-N fertilization is often used to limit vertical leaf growth and increase ball roll distance (green speed).

So, the possibility of following turfgrass nitrogen status in real time has practical management uses to adjust nitrogen fertilization on turfgrass requirements and also to improve turfgrass quality. Until now, few tools are available and they are often subjective. Turfgrass nitrogen status is often measured on a visual color scale or estimation of clipping yield. Nitrogen leaf content analysis give reliable information on turfgrass nitrogen needs but requires more time and money. In this way, diagnostic tools are needed to manage nitrogen treatment.

Turfgrass chlorophyll leaf content has been shown as an indicator of turfgrass nitrogen status that offers a possibility to manage nitrogen fertilization (Mangiafico et al, 2005).

FORCE-A’s Multiplex fluorescence sensor measures in real time chlorophyll and polyphenolics (Agati and al., 2008; Cerovic and al., 2008) content in turfgrass leaves. Polyphenolics content depends on environmental conditions and polyphenolics synthesis increases with stress on plants. Cartelat and al. (2005) have shown that polyphenolics (flavonoïds in particular) content is an indicator of wheat nitrogen status, especially associated with chlorophyll content.

First, this study will demonstrate that chlorophyll and flavonoïds content can be used for turfgrass nitrogen status assessment and that the chlorophyll-to-flavonoïds ratio is a better indicator than chlorophyll alone. Then, this study will illustrate the applicability of Multiplex measurement to optimize turfgrass quality spatially and in time.

MATERIALS AND METHODS

Multiplex sensor

Multiplex is a hand-held multi-parametric fluorescence sensor which can be used in daylight on plants. It has three wavelength light sources (LED): UV_A (375 nm) (UV), green (530 nm) (G) and red (630 nm) (R) and three filtered synchronized detectors for fluorescence recording: blue-green fluorescence (447 nm) (BGF), red fluorescence (665 nm) (RF) and far-red fluorescence (735 nm) (FRF). For a homogeneous and strong enough light on samples, Multiplex has six UV sources and three LED-matrice of green and red light on a circle (figure 1).

Measurement distance is ten centimeters from light sources and the measurement surface is an eight centimeters diameter circle.

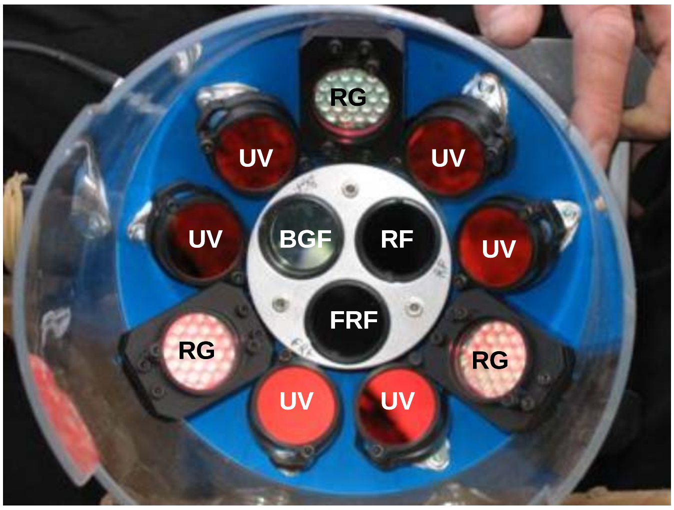


Fig. 1. Multiplex excitation sources and detectors layout

Multiplex emits successively a flash from each excitation source and associated fluorescence emission is registered on each detector. A measurement consists in the average of fluorescence from five hundred individual flashes and takes less than one second. Nine signals (3 excitations x 3 detectors) are available for each measurement. Fluorescence ratios are computed from these signals and give information on chlorophyll, flavonoïds and nitrogen content. Ratios are preferred over single signals because they are less dependant on distance of measurement.

The Multiplex chlorophyll index, SFR, is defined as the simple fluorescence emission ratio FRF_R/RF_R (far-red fluorescence and red fluorescence, respectively, under red excitation). This index is based on an increasing chlorophyll fluorescence re-absorption at its shorter wavelength peak when chlorophyll content increases (Lichtenthaler and al., 1998; Gitelson and al., 1999; Buschmann and al. 2007). The Multiplex flavonoïds index, FLAV, is defined as log(FRF_R/FRF_UV) (far red fluorescence under red and UV excitation respectively). Flavonoïds are UV absorbers in leaf epidermises. To assess flavonoïds content, chlorophyll fluorescence under UV and red light is measured. UV light is absorbed by flavonoïds while red light is not. So, the difference of fluorescence intensity permits to evaluate flavonoïds absorbance (Cerovic and al., 2002). The NBI, Nitrogen Balance Index, is the chlorophyll-to-flavonoïds ratio computed by the ratio FRF_UV/RF_R (far-red fluorescence under UV excitation and red fluorescence under red excitation. This index is based on the balance between primary and secondary metabolism of plants under nitrogen control (Cartelat and al, 2005). Under nitrogen deficiency, chlorophyll synthesis decreases whereas flavonoïds synthesis increases.

The NBI as an indicator of turfgrass nitrogen status

NBI variation with nitrogen fertilization

This trial was carried out on the sprig nursery of Saint-Nom-La-Bretèche (France) golf course. The sprig nursery has been seeded at autumn 2008 on a sandy soil and was composed of creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*). Plots size measured 5 x 10 m. Five nitrogen treatments were applied on to plots: three plots received liquid nitrogen fertilizer at 10 kg N ha^{-1} $month^{-1}$, 20 kg N ha^{-1} $month^{-1}$ and 40 kg N ha^{-1} $month^{-1}$ respectively, another plot received a slow-release granular fertilizer at 20 kg N ha^{-1} $month^{-1}$ and the last one was the untreated control (table 1.).

Table 1. Nitrogen treatments on each plot

Treatment	Nitrogen quantity, kg N ha^{-1}		
	29/04/2009	15/05/2009	03/06/2009
Control	0	0	0
Liquid fertilizer 10 kg N ha^{-1} $month^{-1}$	5	5	10
Liquid fertilizer 20 kg N ha^{-1} $month^{-1}$	10	10	20
Liquid fertilizer 40 kg N ha^{-1} $month^{-1}$	20	20	40
Granular 20 kg N ha^{-1} $month^{-1}$	30	0	20

Multiplex was mounted on a wheeled board to measure turfgrass continuously (fig. 2.). Six rows of 10 meters were measured spaced out by 0.5 meters on each plot. Plots borders were not measured. Around hundred measurements were registered for each plot at five dates. Visual turf density and color ratings were recorded at each date. A comparative rating scale of 1 to 5 was used: 1 for the lowest and 5 for the highest.

Relationship between nitrogen leaf content of turfgrass and NBI.

First experiment: this study was conducted in 2008 and 2009 on a perennial ryegrass (*Lolium perenne*) and annual bluegrass (*Poa annua*) turfgrass from soccer playgrounds of Yves du Manoir Stadium, Colombes (France). In 2008, measurements were performed on seven plots on the soccer playground. Plot size was 3 x 5 m. Different nitrogen rates from 0 to 80 kg N ha^{-1} were applied on each plot. Multiplex was mounted on a wheeled board to measure turfgrass continuously. Six rows of 5 meters were measured spaced out by 0,5 meters on each plot. Turfgrass leaves were collected on each plot and dried at 60°C during 48 hours. Then, the dried leaves were ground to powder. Samples of 5 mg powder (exact weight of each sample was recorded) were sent to a laboratory to identify total nitrogen leaf content for each plot. Nitrogen standard samples (atropine, 4.83 % N) were also sent to evaluate nitrogen leaf content analysis quality. Measurement mean error was evaluated at 0.11 %. Same measurements have been conducted on three other soccer playgrounds with different nitrogen fertilization. Plots were measured on 28/08/2008 and the soccer playgrounds on 28/08/2008 and 25/09/2008. In 2009, measurements were taken on the same three soccer playgrounds only on 30/06/2009 and 15/09/2009, but nitrogen content analyses were carried out by another laboratory on 2 g powder samples. Measurement mean error was 0.03 %.

Fig 2. Multiplex mounted on a wheeled board

Second experiment: this study was conducted in 2009 on greens of five golf courses and on a sprig nursery of another golf course near Paris. Turfgrass was a mixture of creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*). Multiplex measurements were carried out on greens (40 measures on each green) and sprig nursery (100 measures) in the same way as first experiment. Clippings were collected on each place, dried, grinded to powder and sent to the laboratory to evaluate total nitrogen leaf content. These measurements were taken on several greens from each site, on one or two dates between 02/07/2009 to 06/11/2009 and on five places from the sprig nursery with different nitrogen fertilization on following dates: 16/06/2009, 21/07/2009 and 08/09/2009.

NBI kinetics to manage nitrogen fertilization

This study was conducted on five greens (greens 1, 2, 6, 10 and 13) of the Dolce Chantilly golf course. The fertilization was the same on every green except green 1 which did not receive the last fertilization (table 2.).

Table 2. Nitrogen fertilization on greens

Date	Nitrogen quantity applied (kg N ha^{-1})
17/02/2009	24
10/04/2009	Not communicated
24/06/2009	40
05/10/2009	42 except on green 1

Multiplex measurements were performed around twice a month from 30/03/2009 to 12/11/2009 except during summer. Forty measures were randomly recorded on each green. Multiplex was mounted on a wheeled board to keep always the same distance from turfgrass.

Multiplex mapping for assessment of sport field heterogeneity

This study was conducted with Multiplex mounted on a wheeled board on a soccer field in Yves du Manoir Stadium, Colombes (France). The soccer field is 100 meters long and 70 meters wide. To map the soccer field, rows of 100 meters were measured and spaced 3 meters apart at a constant speed of 3 km/h. Each measurement was geo-localized thanks to the Multiplex GPS module. Geostatistical software Surfer was used to analyze the spatial structure of data. Krigging method was used for interpolation and a grid generated. Measurements were performed on 18/02/2010 and 30/03/2010. Between these two dates,, nitrogen was applied on the soccer field.

RESULTS AND DISCUSSION

The NBI as an indicator of nitrogen status of turfgrass

NBI variation with nitrogen fertilization

Table 3. Visual turfgrass color and density comparative ratings for each treatment (D=density, C=color)

Treatment	28/04/09		05/05/09		19/05/09		02/06/09		16/06/09	
	D	C	D	C	D	C	D	C	D	C
Control	1	1	-	1	1	1	1	1	1	1
Liquid 10 u. N	1	1	-	2	2	2	2	2	2	3
Liquid 20 u. N	1	1	-	2	3	2	3	3	3	3
Liquid 40 u. N	1	1	-	3	4	4	4	4	5	5
Granular 20 u. N	1	1	-	3	4	3	3	2	5	4

At the beginning of the trial, no visual differences could be observed between plots. After the first treatment (29/04/2009), differences were observed between treated plots and control. For the liquid fertilizer, three nitrogen rates were applied. An increase in turfgrass density and color was observed with increasing nitrogen amount while the control kept the same aspect. A slow-release nitrogen granular fertilizer was also studied to compare both forms of nitrogen fertilizer usually used on golf courses. An increase in turfgrass color and density similar to the 40 kg N ha^{-1} liquid treatment was observed with the granular fertilizer after the first application. After a month, turfgrass color and density had decreased and looked similar to the 10 kg N ha^{-1} liquid treatment. Second application led to an increase in turfgrass color and density again.

Multiplex measurements were performed at five dates. From these measurements, kinetics of SFR, FLAV and NBI were drawn for each treatment. Each point is the average of measures obtained on each plot and error bars correspond to confidence interval. Error bars size is smaller than symbol size. Arrows represent nitrogen applications, thin = half amount, heavy = entire amount (figure 3.).

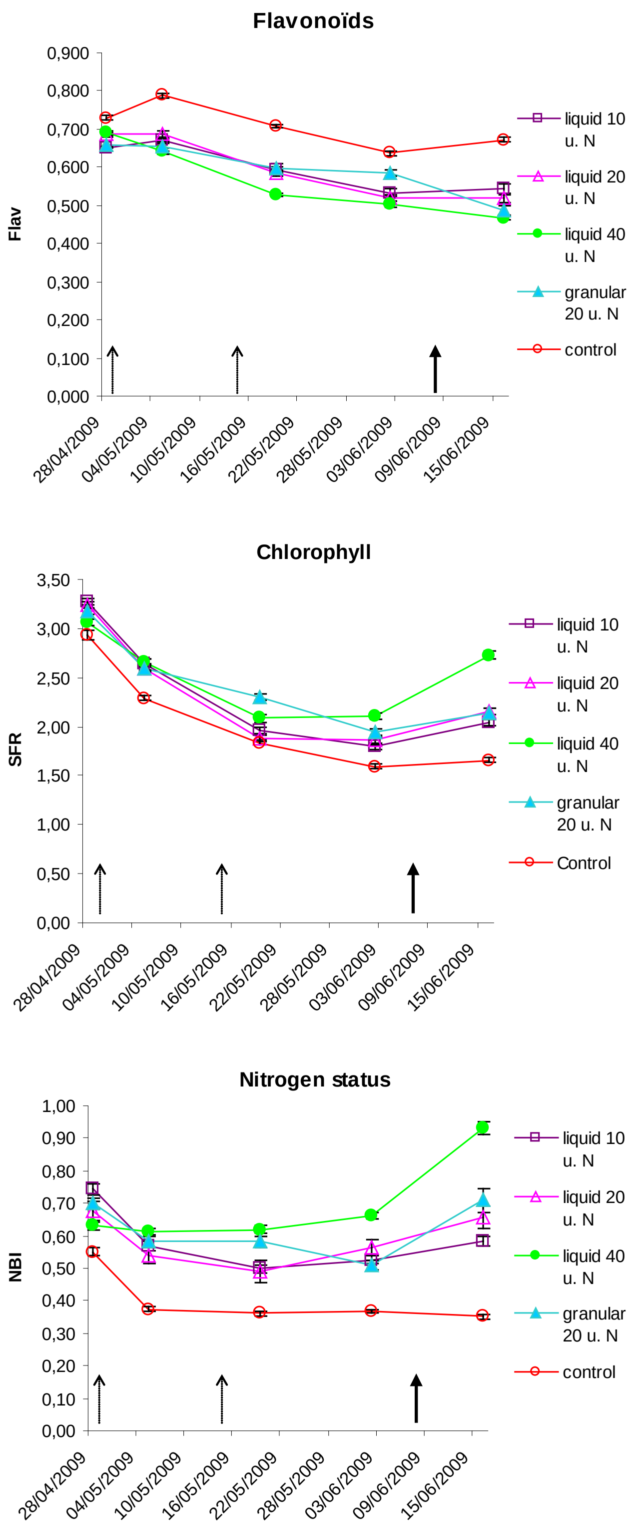


Fig 3. Evolution of SFR, FLAV and NBI on each plot after treatment

After the first nitrogen application, a general decrease in SFR and NBI Multiplex indicators is observed including the control (fig 3.). NBI should have increased due to nitrogen supply. The decrease of SFR and NBI and the increase of FLAV on the control show that other factors than nitrogen fertilization are responsible in optical indicators variations at this moment of the study. So, there is a problem with the measurement of flavonoïds and chlorophyll at the first date. After the first point, SFR, FLAV and therefore

NBI seem to be linked to nitrogen amount. Low light during winter period could have influenced FLAV and SFR at the first date. The turfgrass cutting height has been also reduced at the beginning of the trial. Both chlorophyll and flavonoïds contents increase along the leaf starting from the base (Cartelat and al. 2005). The chlorophyll-to-flavonoïds ratio permits to alleviate partially the problems of gradients along the leaves. It could explain why NBI keeps stable from the second date while FLAV and SFR decrease. Moreover, turfgrass was recently established, so its nitrogen needs were important. Maybe half-rate inputs were not enough to cover nitrogen turfgrass needs.

After the second nitrogen application, NBI increases in relation to nitrogen rate whereas NBI on control keeps stable (fig 3.). In fact, the higher the nitrogen amount applied, the higher NBI. Furthermore, a strong decrease in NBI is observed with the granular fertilizer. This result is well correlated with visual observations (table 3.).

At the third nitrogen application, nitrogen amounts are doubled compared the previous application. Increases in NBI are more important than after second nitrogen application and show NBI positive dependence on nitrogen nutrition level (fig 3.). At this point, NBI levels on each treatment are well correlated with visual ratings (table 3.). SFR also increases with increasing nitrogen amount while FLAV decreases but no difference can be observed between liquid fertilizer at 10 kg N ha^{-1} , liquid fertilizer at 20 kg N ha^{-1} and granular fertilizer with the chlorophyll index SFR whereas visual observations show differences.

So, flavonoïds and chlorophyll are both indicators of turfgrass nitrogen status. The chlorophyll-to-flavonoïds ratio, NBI, accentuates the difference among level of turfgrass nitrogen content because of the chlorophyll and flavonoïds inverse dependence on turfgrass nitrogen status. NBI is more accurate than chlorophyll alone to evaluate turfgrass nitrogen status. Indeed, NBI differences between treatments are better correlated to visual observations than SFR or FLAV.

Relationship between nitrogen leaf content of turfgrass and NBI.

First experiment: Multiplex measurements of SFR, FLAV and NBI obtained on soccer fields in 2008 and 2009 were averaged for each plot and compared to leaf nitrogen content from samples collected on corresponding plot.

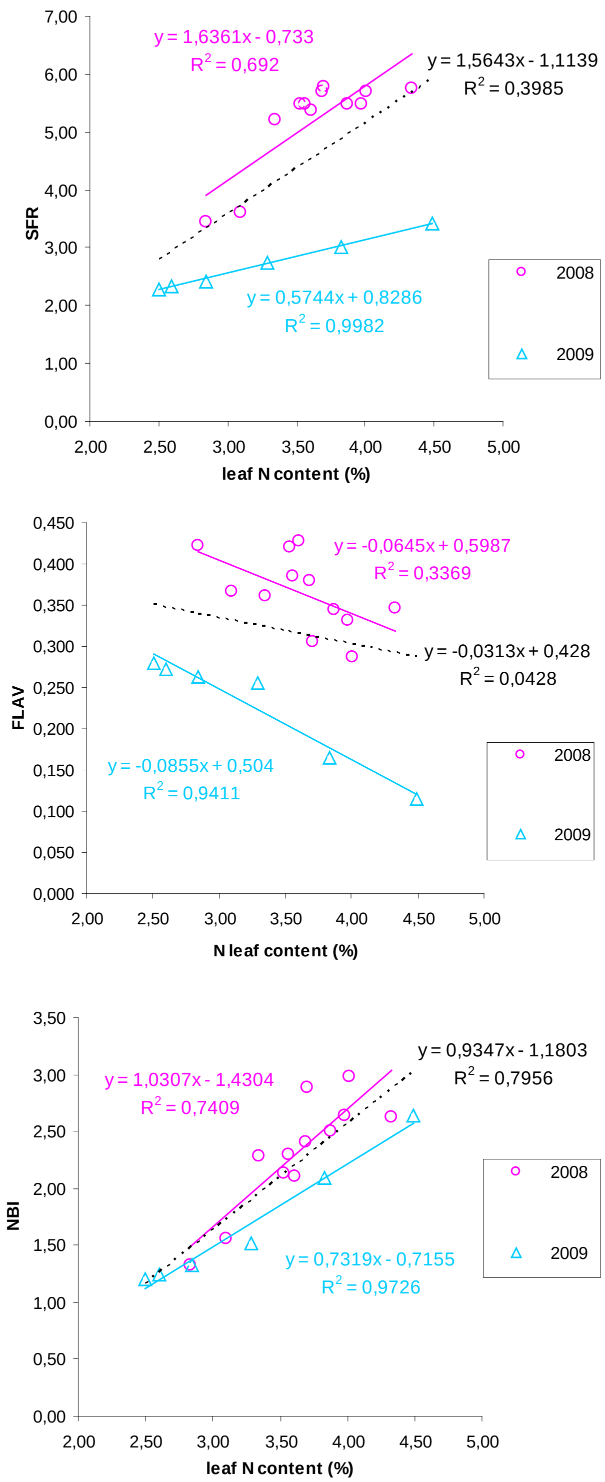


Fig 4. Relationships between SFR, FLAV, NBI and turfgrass leaf (ryegrass and annual bluegrass) leaves nitrogen content from soccer fields in 2008 and 2009

The chlorophyll content index SFR is positively linked to leaf nitrogen content whereas flavonoïds content index FLAV is negatively linked to leaf nitrogen content (figure 4.). However, relationships with leaf nitrogen content are much worse if the two seasons are mixed for both indicators (SFR, r^2=0.40; FLAV, r^2=0.04). Relationships between leaf nitrogen content and these optical indicators are influenced by the year of measurement.

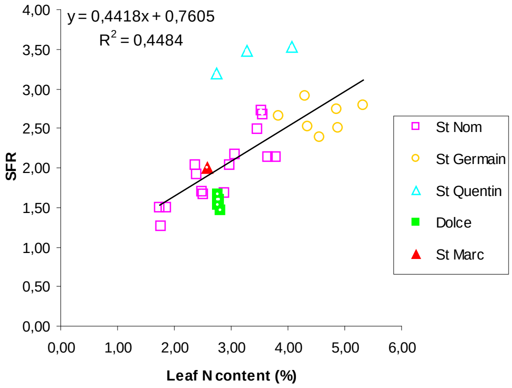
Differences between 2008 and 2009 could be turfgrass cutting height, ryegrass and annual bluegrass proportion, climate, nitrogen analyses laboratory.

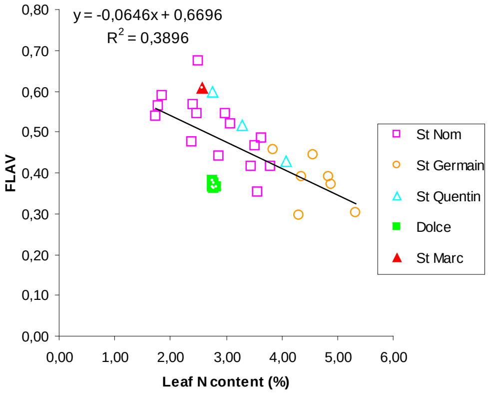
The chlorophyll-to-flavonoïds ratio NBI is highly (r^2=0.8) positively correlated with leaf nitrogen content. The ratio NBI alleviates partially effects of different years.

So, NBI is a good indicator of turfgrass leaves nitrogen content, more reliable than chlorophyll or flavonoïds alone.

The perennial ryegrass leaf nitrogen concentration is given for healthy turf from 3.3 to 5.1 % (Mills and Jones, 1996). These concentrations are also used by turfgrass experts to evaluate nitrogen needs on soccer playgrounds. So, a 3.3 % leaf nitrogen content corresponds to a NBI value around 1.9 (fig 4.). This NBI value could be use as a reference below which soccer field turfgrass is under nitrogen deficiency.

Second experiment: Multiplex measures SFR, FLAV and NBI obtained on golf courses in 2009 were also averaged for each green or plot and compared to leaf nitrogen content from clippings collected on corresponding plot.





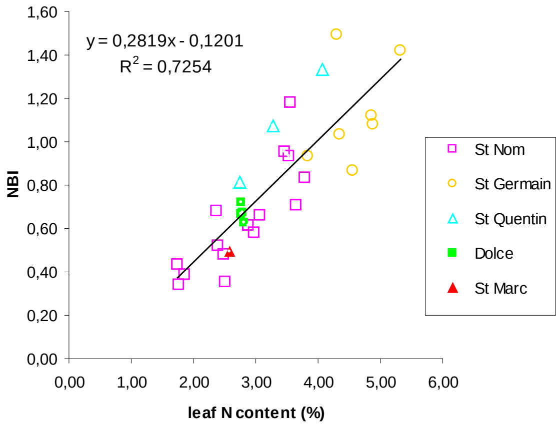


Fig 5. Relationships between SFR, FLAV, NBI and turfgrass (creeping bentgrass and annual bluegrass) leaf nitrogen content on greens of golf courses in 2009.

Positive correlation for chlorophyll index SFR and negative correlation for flavonoïds index FLAV with nitrogen leaf content were also observed. NBI is still highly positively correlated with leaf nitrogen content, better than chlorophyll or flavonoïds alone (SFR, r^2 = 0.45; FLAV, r^2=0.39 and NBI, r^2=0.72) (fig 5.).

This result confirms that NBI is good indicator for assessing turfgrass nitrogen nutrition level, better than chlorophyll alone. This good correlation was obtained despite the fact that different sites, different soil types, different turfgrass species proportions were mixed. This relationship needs to be confirmed for other years.

Relationships between NBI and turfgrass leaf nitrogen content are different for soccer field turfgrass and golf course turfgrass. Differences in turfgrass species (ryegrass for soccer fields and creeping bentgrass for golf courses) and in cutting height (around 40 mm for soccer fields, around 3 mm for golf courses) could explain the difference in absolute values for the NBI to leaf nitrogen content relationships.

NBI kinetics to manage nitrogen fertilization

NBI kinetics were drawn from measurements on five greens of Dolce Chantilly golf course during year 2009. Each point corresponds to the average of forty measures on each green and error bars correspond to confidence interval. Arrows represent nitrogen applications (fig 6.).

A NBI of 0.8 has been defined as a nitrogen deficiency limit on greens thanks to greenkeepers observations of turfgrass color, clipping yield, regrowth capacity and game quality on three other golf courses.

At the beginning of the study, nitrogen status was weak on all greens (fig 6.). During winter period, turfgrass stopped to absorb soil nutrients because of low temperatures and a lack of light. So turfgrass nitrogen status was usually low at the beginning of spring period.

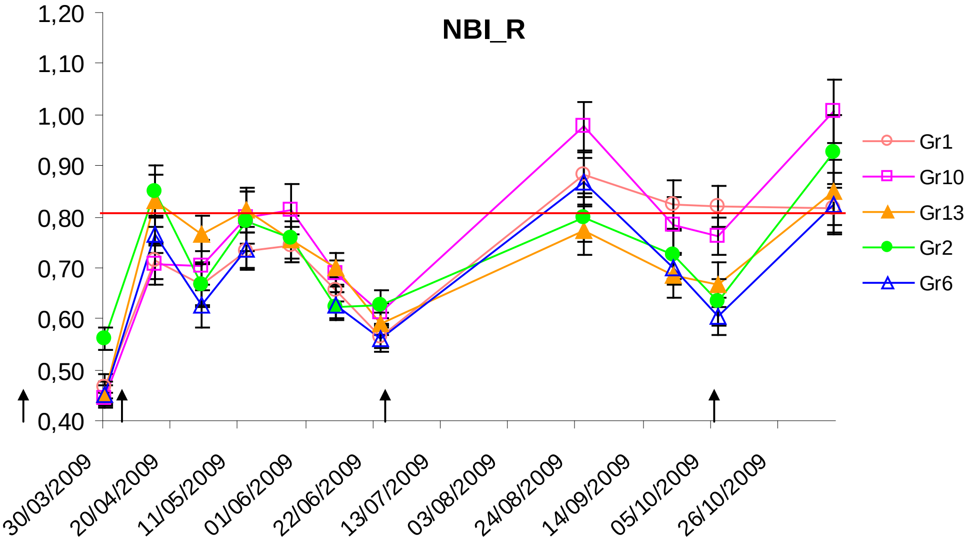


Fig 6. NBI kinetics on five greens from Dolce Chantilly golf course during year 2009

From 30/03 to 15/04, NBI increased on every green due to nitrogen fertilization on beginning of April and better climate conditions. Nevertheless, NBI was still weak particularly on greens 1, 6 and 10. From 15/04 to 27/05, NBI kept quite stable on every green. A slow-release nitrogen fertilizer was used. It allowed a regular nitrogen delivery to turfgrass and so, a stable nitrogen status during several weeks. During this period, NBI was around 0.8 on green 13. NBI was around 0.7 on greens 1, 2, 6 and 10. So nitrogen fertilizer could have been applied on higher quantities on these greens. Small decrease of NBI on 29/04 could be explained by low temperatures around this period that could have reduced nitrogen absorption.

From 25/04 to 24/06, important decrease of NBI was observed. Fertilizer nitrogen amount had been totally used by turfgrass and soil delivery was not enough anymore to cover turfgrass needs. A new nitrogen application could have been done on beginning of June to avoid important nitrogen status decrease.

A new fertilization at beginning of July permitted a strong increase in NBI until end of August. At this moment, greens were neither under nitrogen deficiency nor too vigourous. Green 10 had a higher NBI than other greens. More mechanical operations had been done on this green that might have improved nutrients absorption and increased nitrogen status.

At the end of August, NBI decreased again, and was soon under 0.8 except for green 1. A new fertilization could have been done in mid September. As green 1 grew more than other greens, the greenkeeper decided not to apply nitrogen to this green at the beginning of October. NBI was actually higher on green 1 at end of September and remained stable during October whereas NBI increased on other greens as a result of the fertilizer application in October. At the beginning of November, NBI was above nitrogen deficiency limit on every green. Green 10 had the highest NBI again probably due to better nitrogen absorption.

Multiplex measurements show a weak nitrogen status on this golf course. This observation was confirmed by the greenkeeper who had chosen a low-N fertilization management for year 2009. A weak nitrogen status can lead to problems of turfgrass density and diseases susceptibility. Additional nitrogen applications could have been done. Greenkeeper agreed with this result and planed to increase nitrogen amount on greens in 2010.

Multiplex allows to follow turfgrass nitrogen status in real time and so to evaluate turfgrass nitrogen needs. Thanks to Multiplex, nitrogen fertilization could be adapted to turfgrass needs to avoid nitrogen deficiency or over-fertilization.

Multiplex mapping for assessment of sport field heterogeneity

NBI ratio has been mapped to assess turfgrass nitrogen status variability on the soccer field for both measurement dates (fig 7.).

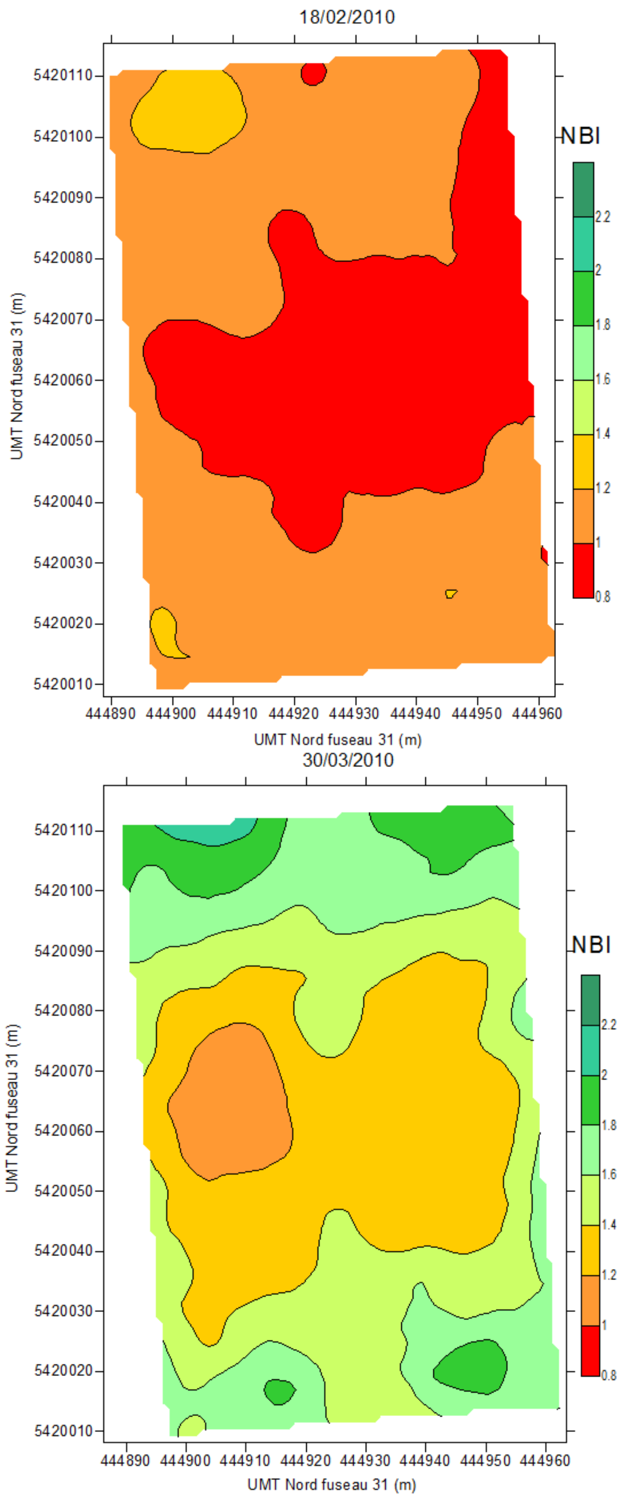


Fig 7. Turfgrass nitrogen status mapping on a soccer field on 18/02/2010 and 30/03/2010

On 18/02/2010, the NBI map shows a weak variability on the soccer field except that the central part where turfgrass nitrogen status is lower. This area is usually more practiced, so turfgrass needs more nitrogen and the ground is more compacted. These factors reduce nitrogen absorption efficiency. On this date, nitrogen status was low because of winter conditions that stop nitrogen absorption and induce stress on turfgrass.

On the second date, the NBI map shows that nitrogen status increased really significantly since the 18/02/2010. Better growing conditions and a nitrogen application between both dates can explain turfgrass nitrogen status increase. The map shows an important variability on the soccer field. Nitrogen status is lower again on the central part of the soccer field. On this zone, turfgrass is under nitrogen deficiency. Turfgrass nitrogen content is the highest on both soccer field edges, particularly in the corners. These areas are less practiced; turfgrass is less pulled up and trodden on, so its nitrogen needs are lower.

NBI maps show that turfgrass is under nitrogen deficiency in the central area of the soccer field whereas its nitrogen status is correct on the rest of it. So, an additional nitrogen fertilization could have been done on the central area of the playground only (which represents around half of the playground area) to improve nitrogen status on this area.

Multiplex can be used to localize areas where turfgrass is under nitrogen deficiency and to adapt nitrogen fertilization on areas where turfgrass needs are higher.

CONCLUSION

Chlorophyll and flavonoïds content in turfgrass leaves are both objective indicators of turfgrass nitrogen nutrition level and give additional information to visual observations. This study has shown that the chlorophyll-to-flavonoïds ratio, NBI, is a better indicator than chlorophyll alone.

NBI allows to assess turfgrass nitrogen status in vivo and in real-time. So, Multiplex is a diagnostic tool that can be used to help nitrogen fertilization management. Multiplex allows to detect nitrogen deficiency but also, by following turfgrass nitrogen status, to apply fertilizer when turfgrass needs it.

Turfgrass nitrogen content mapping on soccer fields allows to adapt nitrogen fertilization to turfgrass needs on a sport field scale.

Multiplex will be soon mounted on lawn mower for online measurements and map production thanks to its GPS module.

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